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CULTURE OF INDICATOR PLANTS IN SOIL FROM COCONUT GROVES AFFECTED BY CADANG-CADANG

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THREE PLATES

ABSTRACT

Research on the cadang-cadang disease of coconuts had been carried out in the Agricultural Research Center, National Institute of Science and Technology, Manila, on the premise that the cause is one which is associated with the soil. This paper is a result of culture studies comparing the performance and characteristics of test plants grown in soils from the Bicol region and Laguna. Test plants were resorted to in this study because the coconut is not only big and unwieldy but it also reacts slowly.

This study seems to indicate that okra is a convenient indicator for the "dark green" response while *Curcuma* is a convenient indicator for the rosetting and malformation.

The very tentative indication on the study with different fractions of the soil extract points to some element in the acid sulfide group as probably linked with cadang-cadang. This aspect of the study may be the subject of a subsequent report.

INTRODUCTION

The cadang-cadang disease of coconut in San Miguel Island, Tabaco, Albay, became alarming in 1926 prompting the manager of the plantation to consult with the U.P. College of Agriculture. Although the observation of Dr. G. O. Ocfemia who was sent by the College to make the study, was rendered soon after his visit to San Miguel, it was not published until 1937. In this report, he seemed to indicate that the disease could be caused by a virus.

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After World War II, more attention was given to the disease because the damage from it was estimated to be mounting. The disease was claimed to have spread from San Miguel to other places in the Bicol region, hence, the strengthened supposition that it could be caused by a virus. The impression that San Miguel was the origin of cadang-cadang persisted, notwithstanding the statements of the old men in the region that disease outbreaks in various places, such as Nabua, and Calolbon, antedated that in San Miguel.

One of the present writers became acquainted with the disease in the course of a trip to the area for the National Committee on the Cadang-Cadang of Coconuts and Mosaic of Abaca (Espino and Velasco, 1950). It was observed that there was an apparent gravity of the disease among the tall and old coconut trees. Since water had to be raised to a greater height among tall trees, the reduction in the crown was taken to mean that there could be a derangement in the water status. Either the water passage was blocked or the capacity of the plant to mobilize water was debilitated. However, the hypothesis was not pursued until much later.

The usual symptoms noted on cadang-cadang were: (*1*) the gradual diminution of the crown; (*2*) the leaves appeared nonsucculent, light and rigid like feather in a badminton shuttle-cock and the angle between the trunk and the old leaves successively increased; (*3*) a general yellowing (actually, orange to bronze appearance) and the presence of tiny olivaceous or water-soaked specks in the older leaves; (*4*) during the mild stage of the disease the leaves tended to be far apart, while in the severe stage, the leaves tended to form a rosette; and (*5*) in the severe stage, the young leaves tended to be thick, malformed, brittle and conspicuously dark-green. The problem was how to determine which of these symptoms was diagnostic.

On further study of the disease, it came to be noted that in some places trees died while barely 5 meters tall, while in other localities coconut trees grew up to 20 meters or more before they succumbed to cadang-cadang. There were also indications that nitrate fertilizer tended to aggravate the disease while phosphate improved the appearance and fruiting of the trees; but once the disease set in, even the phosphate fertilizer did not stop deterioration and death. These indications could implicate the soil in the cadang-cadang problem. Approaching the problem from the soil angle, the following hypothesis may be adopted: (*a*) the soil might be deficient in some essential element, (*b*) it might contain an excess of some constituent so that the balance of nutrients became upset, or (*c*) it might have an element, which is toxic even in low concentration.

This paper is a result of culture studies comparing the performance and characteristics of plants grown in soil from the Bicol region and those grown in Laguna soil. The object of the studies were to find an indicator plant for use in the further study of cadang-cadang, and to gain some indication on the nature of the soil constituent which may cause the disease.

The work stretched over many years but most of the data in the paper were gathered in 1969-1971, from trials made in the National Institute of Science and Technology.

MATERIALS AND METHODS

1. *The soil samples.*—Soil samples were taken from Batang, Ligao; Banao, Guinobatan, Bankerohan, Daraga, and San Miguel Tabaco — all in Albay Province. Among the places sampled, cadang-cadang was found most mild in Bankerohan; the rest were heavily affected with the disease. A sample was taken from Maahas, Los Baños, Laguna to represent the nonaffected area. Sampling was done at the surface around the coconut trees up to 2 meters away from the trunk and up to a depth of 8 inches.

2. *The test plants.*—The coconut groves in Laguna, Quezon and Bicol provinces were surveyed to note the plant species thriving well in Laguna and Quezon but unhealthy in the Bicol region; likewise, the plants present in Laguna and Quezon but absent in the Bicol provinces. Many of the species used in the experiments were chosen from the list compiled in this survey.

Among the species tried were *Phaseolus aureus, Vigna sinensis, Zinnia elegans, Brassica* sp., *Ipomoea triloba, Helianthus annuus, Tagetes erecta, Cosmos caudatus, Lycopersicum esculentum, Capsicum frutescens, Portulaca oleracea, Coleus blumei, Lemna minor, Tridax procumbens, Solanum melongena, Allium cepa, A. sativa, Tectona grandis, Lagerstroemia speciosa, Passiflora foetida. Melastoma polyandra, Synedrella nodiflora, Ageratum conyzoides, Vernonia cinerea, Stachytarpheta jamaicensis, Emilia sonchifolia, Gossypium hirsutum, Ceiba pentandra, Bixa orellana, Abelmoschus esculentus, Catharanthus roseus, Amaranthus viridis, A. spinosus, Phaseolus vulgaris;* and *Curcuma zedoaria.*

Because tests with most of the species did not yield distinctive observations, only the data concerning *Abelmoschus esculentus* (okra) and *Curcuma zedoaria* (barak), an uncultivated relative of the turmeric plant, are presented.

3. *Method of culture.*—Cultures were run in triplicate.[1] The plants were grown in about 2 kg of soil (or sand) in earthen pots. Soil from Ligao was taken to represent the cadang-cadang affected region and soil from Los Baños (Barrio Maahas), to represent the nonaffected region. To insure that the lots were provided with comparable nutrition, they were watered regularly wih Hoagland's solution.

In studies with soil extracts and their fractions, the plants were grown in sand and started with Hoagland's solution. Subsequently, the extracts were incorporated with Hoagland's solution at concentrations up to 1,000 parts per million, using as basis of computation the residue left when a hydrochloric acid solution of the extract (or fraction) is evaporated to dryness.

Differences in growth and general appearance of the plants in the cultures were noted.

EXPERIMENTS AND RESULTS

1. *Comparison of okra grown in various soil samples.*—Okra seeds were germinated in sand and as soon as the cotyledons became expanded, usually 5 days after sowing, the seedlings were transferred in soil samples from Maahas (Los Baños), San Miguel, Guinobatan, Bankerohan, and Ligao. The latter four soil samples were from cadang-cadang affected coconut groves. At the end of five weeks, representative plants were photographed (Plate 1, fig. 1). Despite the equalizing effect of Hoagland's solution used in watering them, they exhibited visible differences. The plant in Maahas (Los Baños) was biggest and its leaves appeared normal. The plants in the affected soil samples were small with cupped-down and distorted

TABLE 1.—*Number of leaves of okra grown in Los Baños and Ligao soils (average of three replicates).*

Age in days from germination	Los Baños	Ligao
7	2.6	2.6
17	6.0	5.3
30	4.6	3.0
37	4.6	2.0

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[1] The assistance of Messrs. Eduardo Mercado and Virgilio Aurelio in running the cultures is gratefully acknowledged.

leaves. The conspicuously dark-green young leaves of plants in the affected soil appear as dark shade.

The periodic leaf counts of Los Baños and Ligao (taken as representative of the affected soils) are presented in Table I.

It may be noted that starting with the 17th day, the plants showed the adverse effect of the Ligao soil in terms of fewer leaves. This became increasingly grave so that at 37 days the number of leaves in Ligao was less than half of that in Los Baños. The difference was due to the faster abscission of old leaves in the Ligao culture coupled with the slower production of young leaves.

In another experiment, comparable series of cultures were conducted in Los Baños and Ligao soils. One set was not fertilized, and three other sets were given ammonium nitrate fertilizer at the rate of 0.5, 1.0 and 2.0 g per pot. As usual, the plants were watered periodically with Hoagland's solution. Representative plants were photographed at about one month after the fertilizer was applied (Plate 1, fig. 2).

The plants in Ligao soil did not improve with the application of ammonium nitrate. On the other hand, the plants in Los Baños soil were distinctly healthy, even without fertilizer — i.e. with Hoagland's solution alone (control). With supplementation of ammonium nitrate, the plants became more vigorous. The leaves seemed progressively broader as more fertilizer was added. This may be taken as one indication that this sample of Ligao soil contains something inhibitory to the growth of okra. The likelihood that some essential element is deficient was minimized because the plants were watered with Hoagland's solution, also because in previous experiments, various essential elements applied as fertilizer were ineffectual.

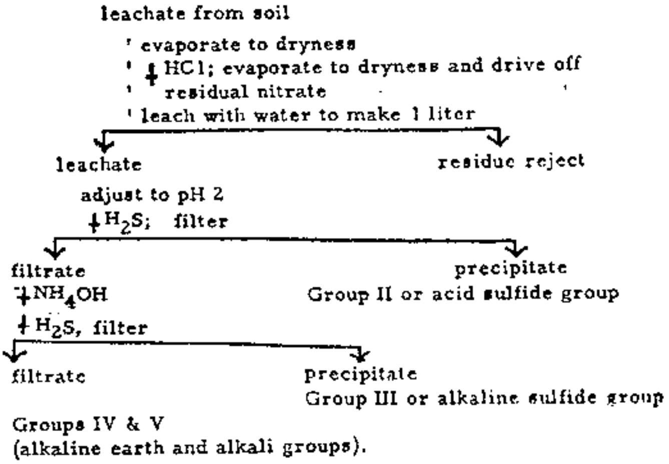
2. *Growth of okra in soil extracts.*—One significant observation in the laboratory was that the affected soil frothed profusely when treated with oxidizing acid (Velasco *et al*, 1968). In some cases samples for analysis got spoiled because they spilled over upon digestion over a flame. One common cause of much frothing under this condition is the presence of high organic matter. Of course the presence of tungsten, molybdenum or selenium may also cause frothing in certain cases.

a. Since humic acid is a principal fraction of organic matter in the soil, it was used in this experiment to gain some idea on the reaction of okra to organic matter. Humic acid was prepared from Ligao and Los Baños soils by extraction with 0.5-per cent sodium hydroxide and neutralization with hydrochloric acid. A weighed quantity of the fresh precipitate was resuspended in a definite amount of water to constitute a stock solution, while a comparable amount was dried at 100°C to determine the oven-dry weight. Using the oven-dry weight as basis of dilution, a 10-ppm solution was made from the stock solution using Hoagland's solution as diluent. This solution was made to trickle on the sand where okra seedlings were growing.

The plants did not differ in growth among the three treatments. If at all, the plants in the Ligao treatment were slightly more vigorous than in the other two treatments up to 60 days of age. Since cadang-cadang is associated with older plants, the cultures continued up to 105 days when the plants had passed their first flush of fruiting. It can be seen in Plate 1, fig. 3 that even at this late date, it was hard to distinguish one treatment from the other. It would seem that the detrimental factor in Ligao soil was not associated with humic acid.

b. In order to observe the effect of the inorganic constituents which can be extracted from the soil, 2 kg of Los Baños soil and Ligao soil were digested with 500 ml of concentrated nitric acid. Then the digestate was leached with 1 liter of water in 500-ml portions. The scheme of separation into groups was essentially that of Hogness and Johnson (1954) as shown in Scheme 1.

SCHEME 1. *Fractionation of elements in soil extract.*


leachate from soil
evaporate to dryness
+ HCl; evaporate to dryness and drive off residual nitrate
leach with water to make 1 liter
leachate
residue reject
adjust to pH 2
+ H_2S; filter
filtrate
precipitate
Group II or acid sulfide group
+ NH_4OH
+ H_2S, filter
filtrate
precipitate
Group III or alkaline sulfide group
Groups IV & V
(alkaline earth and alkali groups).

The sulfide precipitates of groups II and III were digested with hydrochloric acid and the solutions were evaporated to dryness. The filtrate representing groups IV and V were acidified with hydrochloric acid and evaporated to dryness. In other words, the residues which were weighed and made the starting material for subsequent dilutions with Hoagland's solution were essentially the chlorides of the elements constituting the groups.

It can be seen in Plate 2, fig. 1 that the plants treated with a supplement of 10 ppm and 100 ppm of group II elements (copper-arsenic) from Los Baños soil did not differ noticeably from the control plants treated with Hoagland's solution alone. On the other hand, the plants treated with a 100-ppm supplement of group II elements from Ligao soil seemed to be more vigorous. It was also noted that the Ligao culture were conspicuously darker green than the others. It is tempting to ask if the suspected toxic element in this group was serving as a stimulant at this level of concentration.

There seemed to be no difference in the growth of okra in the other groups of elements (Plate 2, figs. 2 and 3).

3. *Curcuma zedoaria as a test plant.*—Canoy and Velasco (1964) called attention to the fact that while this plant abounded in coconut plantations in Laguna and Quezon, it was rarely met in groves affected by cadang-cadang. However, nothing further was done about this observation, until May of this year when it was tried as a test plant.

The plants in Ligao soil had an early start and appeared more vigorous than those in Los Baños soil. But when the plants were 68 days old, the plants in Ligao seemed to deteriorate. It will be noted in Table 2 that the Ligao plants were distinctly taller than the Los Baños plants at 68 days old. The leaves of the former were long

TABLE 2.—*Periodic measurements of Curcuma in Ligao and Los Baños soil (planted May 12, 1971; average of three replicates).*

Age in days	Soil		Measurement in cm of recently exserted blade		Number of leaves
	Culture medium	Height			
		cm	*Length*	*Width*	
68	Ligao	59.7		11.6	5.6
	Los Baños	54.0		13.3	6.0
168	Ligao	57.6	30.6	10.6	3.3
	Los Baños	63.0	35.2	10.6	4.0
196	Ligao	51.4	25.0	8.2	3.3
	Los Baños	60.5	29.0	8.6	4.0

All measurements were on the mother plant. At the last observation Ligao (1) had two suckers, (2) four suckers, (3) one sucker; Los Banos (1) no sucker, (2) one sucker, (3) two suckers.

and narrow and at this age, the old leaves tended to dry up faster than the Los Baños plants.

At 168 days old, the Ligao plant became distinctly shorter and the leaves smaller. They tended to form a rosette — the youngest leaf hardly getting exserted from the cluster of leaf-sheaths. The dwarfing and deterioration was further aggravated at 196 days old.

Plate 3, fig. 1 shows the plants at 111 days old when it was noticed that the Ligao plants exhibited severe water-soaking. The occurrence was preceded by three rainy days and before then, 19 days of fairly dry weather.

DISCUSSIONS

The work on cadang-cadang has been at a very slow pace because there has not been a convenient test plant. As a test plant, the coconut is rather unsatisfactory not only because it is big and unwieldy but also because it reacts slowly. The earliest that a coconut could die of cadang-cadang is claimed to be 10 years old and the latest is some 50 to 70 years old. It is a long period of observation and waiting before some evidence could be gained for, or against a hypothesis. At the same time it is recognized that for any postulated symptom of cadang-cadang to be meaningful, recourse has to be made on the coconut. Because of this, the work has shifted from the coconut to test plants and back. In many cases, this has been a continuous, unfruitful shuttle.

Among the characteristics observed, the gradual diminution of the crown due to a rapid drying up of old leaves (although quite consistent) may not be considered a diagnostic symptom because many factors other than cadang-cadang may bring it about. Again, the general yellowing and the presence of olivaceous or water-soaked specks may be neglected because they are not consistent and, apparently, are contingent on a mixture of climatic conditions. On the other hand, the conspicuously dark-green young leaves seem to be more consistent and deserve further attention. In the severe stage, the dried up or nonsucculent appearance and the distortion of young leaves seem to be quite diagnostic. It may be worthwhile to select indicator plants which show these characteristics when grown in affected soil. This study seem to indicate that okra is a convenient indicator for the "dark-green" response, while *Curcuma* is a convenient indicator for the rosetting and malformation. In the study of Martin and Trelease (1938), the dark-green young leaves in tobacco were elicited by selenium in the root medium. Conceivably, a few other toxic substances can bring about the dark-green coloration in the young leaves. We are not as yet aware of any study which has associated rosetting with the presence of a toxic substance in the soil medium.

The very tentative indication on the study with different fractions of the soil extract points to some element in the acid sulfide group as probably linked with cadang-cadang. Some of the rather toxic elements happen to fall in this group. Among them are tungsten, molybdenum, mercury, copper, cadmium, arsenic, antimony tin, the platinum metals and selenium.

In our attempt at fractionating the elements into groups, we make it a point to start with their chlorides because the chlorides are most soluble. Also, the chlorides (unlike the nitrate) are not oxidative; hence, it would not oxidize hydrogen sulfide to precipitate elemental sulfur. Unhappily, the chlorides of some members of the acids sulfide group, like arsenic chloride are volatile at fairly low temperatures and are likely to be lost in the course of evaporating a solution or baking a residue. We tend to suspect that this was the case in our preparation of the acid sulfide group. In the Ligao extract, the apparent stimulating effect of this group at 100 ppm could indicate that the toxic level had not been reached. The usual observation is that most toxic elements are stimulating at very low level.

In the counterpart study on the isolation and identification of the putative toxic element the use of hydrochloric acid was avoided. Instead, the soil was extracted with nitric acid and sulfuric acid. The excess nitrate was driven off before treating the extract with hydrogen sulfide. This study may be a subject of a subsequent report.

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ILLUSTRATIONS

PLATE 1

FIG. 1. Okra grown in Maahas (Los Baños) in Bicol soils.

2. Okra grown in Maahas (Los Baños) and in Ligao soil, fertilized with various amounts of ammonium nitrate (Planted November 13, 1969; fertilized November 21 and photographed December 18).

3. Okra treated with humic acid extracts from Ligao and Los Baños (Maahas).

PLATE 2

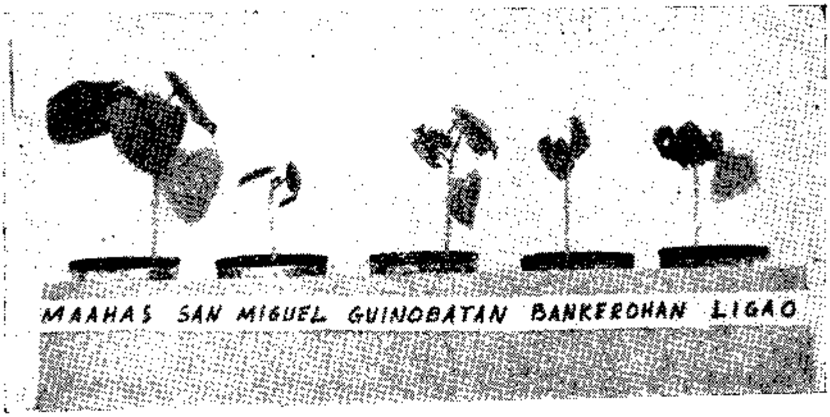
FIG. 1. Growth of okra in Hoagland's solution supplemented with the chlorides of group II, copper-arsenic group.

2. Growth of okra in Hoagland's solution supplemented with the chlorides of group III, iron-manganese group.

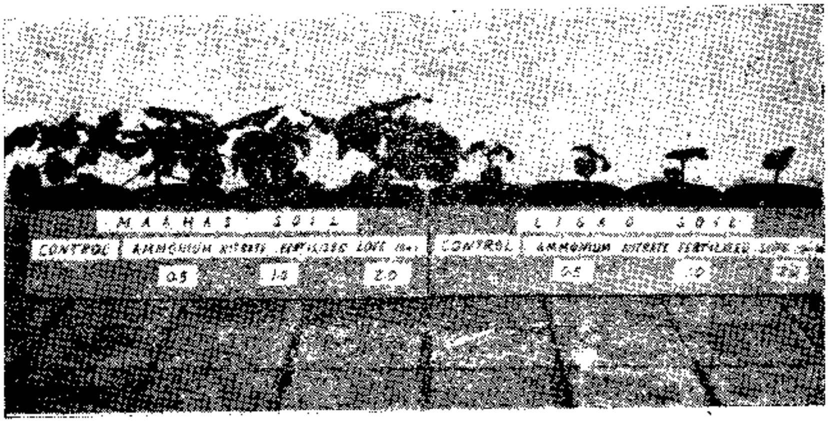
3. Growth of okra in Hoagland's solution supplemented with chlorides of groups IV and V, alkaline earth and alkali group.

PLATE 3

FIG. 1. *Curcuma* plants at 111 days old.


MAAHAS
SAN MIGUEL
GUINOBATAN
BANKEROHAN
LIGAO

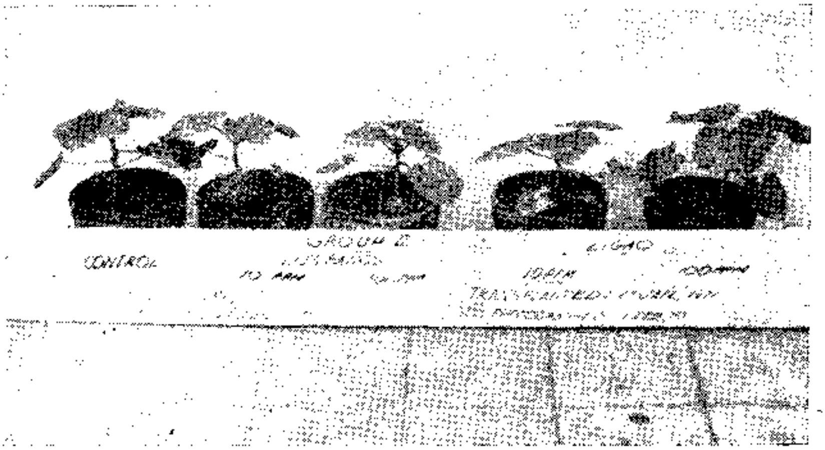
1


MAAHAS SOIL
CONTROL
AMMONIUM NITRATE FERTILIZER LEVEL
0.5
1.0
2.0
LIGAO SOIL
CONTROL
AMMONIUM NITRATE FERTILIZER
0.5
1.0
2.0

2

3

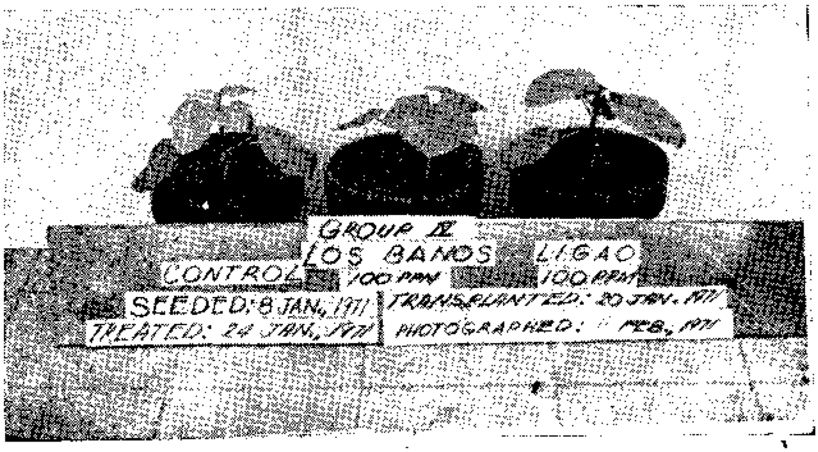
PLATE 1.


GROUP II
CONTROL
LOS BANOS
10 PPM
LIGAO
10 PPM
100 PPM
TRANSPLANTED

1

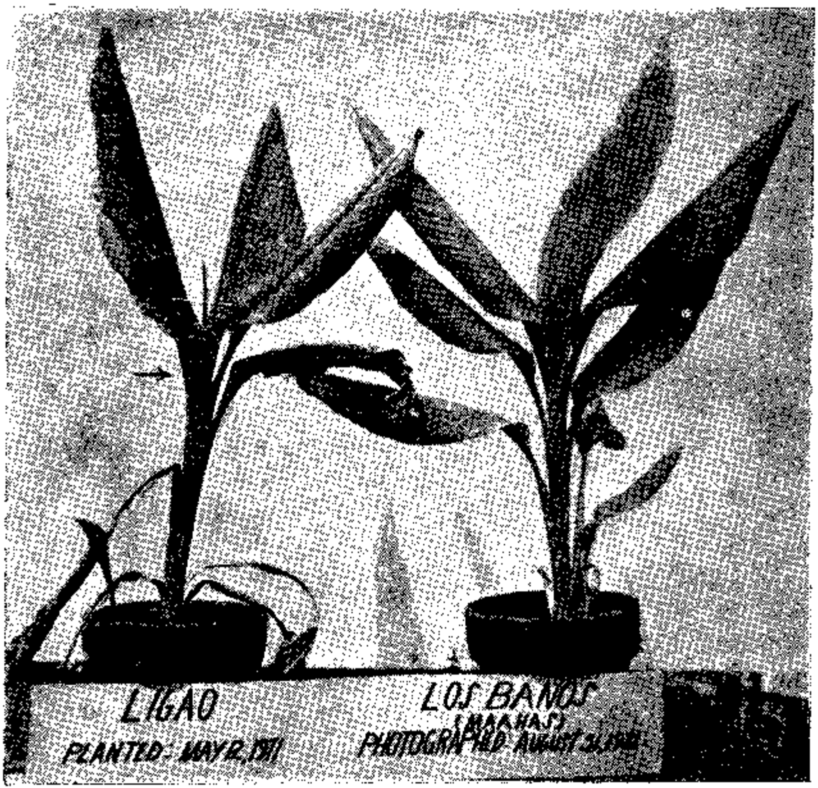
CONTROL
MAAHAS EXTRACT
10 PPM
100 PPM
LIGAO EXTRACT
10 PPM
100 PPM
SEEDED: 19 OCT. 70
TRANSPLANTED: 27 OCT. 70
TREATED: 2 NOV. 70
PHOTOGRAPHED: 23 NOV. 70

2


GROUP IX
LOS BANOS
LIGAO
CONTROL
100 PPM
100 PPM
SEEDED: 8 JAN., 1971
TRANSPLANTED: 20 JAN., 1971
TREATED: 24 JAN., 1971
PHOTOGRAPHED: FEB., 1971

3

PLATE 2.


LIGAO
LOS BANOS
(MAAHAS)
PLANTED: MAY 12, 1971
PHOTOGRAPHED: AUGUST

1

PLATE 3.

THE AVAILABILITY OF CAROTENE IN SOME PHILIPPINE VEGETABLES, II

MUSTASA, GABI LEAVES, SALUYOT AND KALABASA TOPS

By ILUMINADA C. ORTALIZA, ISABEL F. DEL ROSARIO,
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ABSTRACT

The dietary source of Vitamin A is either preformed Vitamin A present in animal foods, or carotene called provitamin A in plants. However, utilization of carotene depends upon its conversion to vitamin A (retinol) in the body. Although theoretically beta-carotene can yield half its weight of vitamin A, availability in practice depends on limited absorption of beta-carotene from foods and its converson to Vitamin A. It is therefore necessary to have information on the availability of carotene in Philippine vegetables used in the preparation of lcoal dietaries. With this aim in view this study was undertaken.

The carotene availabilities from *mustasa; gabi* leaves, *saluyot* and *kalabasa* tops were estimated by the liver storage test using albino rats as the experimental animals. The choice of these vegetables was based on the high carotene content.

The data obtained here were compared with *malunggay, petsay, kangkong* and *kamote* tops in a similar experiment previously undertaken.

Nutrition surveys conducted by the FNRC in eight regions of the country revealed that vitamin A was one of the nutritional deficiencies observed among Filipino.[1] The dietary source of vitamin A is either preformed vitamin A present in animal foods, or carotene called provitamin A in plant foods. Regional nutrition surveys in the Philippines pointed out that 87 per cent of vitamin A value in the diet is in the form of carotene.[2] However, utilization of carotene depends upon its conversion to vitamin A (retinol) in the body. Although theoretically beta-carotene can yield half its weight of vitamin A, availability in practice depends on limited absorption of beta-carotene from foods and its conversion to vitamin A. Therefore information on the availability of carotene in Philippine vegetables is necessary in the preparation of local dietaries.

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[1] E. S. Quioque, G. M. Villiavieja, and V. Ramos. Summary Results of the Eight Regional Survey Conducted in the Philippines by the Food and Nutrition Research Center, NIST, NSDB, Part I—Dietary Phase; Phil. Jour. Nutr. **2**: 61, 1969.

[2] C. Ll. Intengan, and Technical Committee Food and Nutrition Research Center, NIST, NSDB. Recommended Dietary Allowances for Filipinos 1970. Revision Phil. Jour. Nutr. April-June XXII **2**: 17, 1970.

In this country, the first investigation along this line was conducted by Ortaliza *et al* (1969). The main objective was to determine the availability of carotene in some Philippine vegetables which is a mesaure of the amount biologically utilized. Evaluation of four vegetables, namely: *malunggay, petsay, kangkong,* and *kamote* tops clearly showed that differences in the availability of carotene from various sources exist [Ortaliza *et al* (1969)]. It was therefore proposed to study other vegetables. This paper presents the results on another set of four leafy vegetables, namely: mustasa [*Brassica integrifolia* (West) O. E. Schultz], gabi leaves (*Calocasia esculentum* Linn.), saluyot (*Corchorus oliterius* Linn.), and kalabasa tops (*Cucurbita maxima* Duch.). These vegetables were selected because of their high carotene content, they are cheap sources of carotene and are commonly eaten by Filipinos. The plan of experiment was patterned after the first report, liver storage being the criterion for assessing carotene availability.

EXPERIMENTAL

Materials. — Samples of *mustasa, gabi* leaves, *saluyot* and *kalabasa* tops were purchased from local markets and dried in a vacuum oven at 60° to 70°C. Samples were then analyzed for beta-carotene and incorporated into the diet as previously described [Ortaliza *et al* (1969)]. The level of 60-mcg carotene daily proved to be satisfactory for the first investigation so it was decided to adopt this level in the present experiment. The diets were compounded so that 5- to 6-g diet contained 60-mcg beta-carotene. Crystalline beta-carotene (100 per cent) dissolved in wesson oil was used as the control. Diets were analyzed for carotene and kept frozen during the entire experiment just like the first experiment.

Test animals. — Animals used in this experiment were 28-day-old albino rats taken from the stock colony of the FNRC. The procedure for depleting the vitamin A reserves of the animals was as previously described [Ortaliza *et al* (1969)]. As in the previous investigation 10 males and 10 females were assigned to a group. Since there were not enough experimental animals available only two vegetables were studied at a time. For comparison a control group receiving 60-mcg beta-carotene daily was maintained for every experiment.

Method. — Details of the experimental method were essentially the same as in the previously reported experiment [Ortaliza *et al*

(1969)]. The kidneys were not collected in this study as they were found in the previous study to contain negligible amounts of vitamin A. Carotene analysis of food samples and diets was done using the chromatographic method.[3] Livers were analyzed for vitamin A using the Carr Price method.[4]

RESULTS AND DISCUSSION

Table 1 shows the composition of the different diets. Table 2 gives the carotene content of vegetables used. Carotene utilization for two experiments in which four vegetables were studied are shown in Table 3.

TABLE 1.—*Percentage composition of the different diets.*

	Experiment I			Experiment II		
	Control	Mustasa	Kalabasa tops	Control	Saluyot	Gabi leaves
Total	100.0	100.0	100.0	100.0	100.0	100.0
Rice flour	62.0	57.0	52.9	62.0	55.8	57.5
Casein	20.0	20.0	20.0	20.0	20.0	20.0
Salt mixture[1]	4.0	4.0	4.0	4.0	4.0	4.0
Vit. Cornstarch[2]	4.0	4.0	4.0	4.0	4.0	4.0
0.5-per cent alphatocopherol in wesson oil	8.0	8.0	8.0	8.0	8.0	8.0
Wesson Oil	2.0	2.0	2.0	2.0	2.0	2.0
Mustasa		5.0				
Kalabasa			9.1			
Gabi leaves						4.5
Saluyot					6.2	

[1] U.S.P. XIV Nutritional Biochemicals Corp.

[2] Kg. cont. vit. K, 100 mg; PABA, 2 g; inositol, 2 g; calcium panthothenate, 1 g; niacin, 1 g; thiamine, 100 mg; riboflavin, 100 mg; pyridoxine HCl, 100 mg; folic acid, 10 mg; biotin, 10 mg.

TABLE 2.—*Carotene content of vegetables.*

Common name	English name	Scientific name	Carotene content
			mg/100 g dry sample[1]
Mustasa	Mustard	*Brassica integrifolia* (West) O.E. Schultz	23.94
Gabi (tangkay at dahon)	Taro (leaves and petioles)	*Colocasia esculentum* Linn.	27.17
Saluyot (talbos)	Jute	*Corchorus olitorius* Linn.	19.36
Kalabasa (talbos)	Squash (young leaves)	*Cucurbita maxima* Duch.	13.25

Moisture content 5.7 per cent

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[3] Methods of Vitamin Assay. 1947. The Association of Vitamin Chemists (2nd ED.) New York: Interscience Publishers, Inc., pp. 52-63, 26-34.

[4] *Ibid.*

Although the manner of depletion of vitamin A reserve is the same in experiments I and II, it was observed that the initial vitamin A values of rat livers in experiment II were all higher than those reported in experiment I. It was noted that rats used for experiment II were of the third litter which probably might have stored more vitamin A as compared to rats used for experiment I which were rats of the first litter. Studies have shown that the length of depletion period depends upon the vitamin A status of the animal [Oser *et al* (1945)]. Another factor which might have contributed to the variable initial values was the fact that the age of the mothers when mated were not the same. For future studies on nutrient utilization, it is suggested that only the first litter rats from females mated at the same age be used.

Based on complete utilization of beta-carotene, the data for liver storage of the vegetables tested were expressed as percentage of that found for beta-carotene. The results shown in Table 3 indicate carotene utilization of *mustasa, gabi* leaves, *saluyot* and *kalabasa* tops to be 53.00, 38.70, 21.43, and 18.82 per cent, respectively.

The vegetables tested in experiments I and II differ significantly from each other in carotene utilization, except *kalabasa* compared with *saluyot*. While the differences in carotene utilization between *kalabasa* tops and *saluyot* were not statistically significant ($<.05$) they do suggest that carotene from *saluyot* is better utilized than that in *kalabasa* tops. Further examination of the results showed that *kalabasa* tops and *saluyot* were considered poor sources of carotene. *Mustasa* was found to have the highest per cent carotene utilization among the vegetables studied in this report.

Results given in Table 3 also showed that the vegetables tested were significantly less efficient sources of vitamin A for liver storage than was beta-carotene in oil. The results of the present investigation are therefore, in accordance with the more generally accepted view that utilization of carotene from the vegetables is much less than that of pure beta-carotene in oil. Similar results were found in the previous study.

The range of carotene availability of the different vegetables tested in this study is between 19 and 53 per cent which is relatively lower than the previously reported investigation which was 32 and 76 per cent [Ortaliza *et al* (1969)]. Comparison of availability values between the first and the second study seems to be justified based on figures from the control group. The mean carotene utilization of the different control groups did not vary too much. Differences

TABLE 1.—*Liver storage of vitamin A from carotene in some local vegetables as compared with carotene in oil.*
(Values are averages for each group of rats.)

	Experiment I			Experiment II		
	Control	Mustasa	Kalabasa tops	Control	Gabi leaves	Saluyot
No. of animals	20	20	20	19	20	19
Initial weight, g	65.0	65.0	65.0	67.0	67.0	67.0
Initial vitamin A (IU)	43.53	43.53	43.53	77.40	77.40	77.40
Final vitamin A (IU)	361.61	212.13	103.41	419.40	209.77	150.70
Vitamin A stored (IU)	318.08	168.60	59.88	342.00	132.37	73.30
Relative carotene availability (per cent)[1]		53.00	18.82		38.70	21.43

[1] $\frac{\text{Stored Vitamin A, exp. diet}}{\text{Stored Vitamin A, control}} \times 100$

among the different control groups were found not significant ($P<.05$). It would therefore appear that a fairly high degree of reproducibility is possible using liver storage as a measure of utilization.

It should be recalled that in the previous investigation, the carotene availabilities of *petsay, kangkong, kamote* tops and *malunggay* were 75.95, 59.26, 42.27, and 32.26 per cent, respectively [Ortaliza *et al* (1969)]. Comparison of these data with those obtained in the present study tend to show that differences in carotene availability among the different vegetables exist. Statistically significant differences were found in the carotene utilization of the majority of the vegetables included in these two studies with the exception of *kamote* tops compared to *gabi* leaves and *mustasa* compared to *kangkong*.

Guggenhein (1944) found that the utilization of carotene derived from various plant materials ranged between 33 and 67 per cent of the preformed vitamin A. A range of 16 to 64 per cent, with the average of 32-per cent carotene utilization on the basis of beta-carotene dissolved in oil taken as 100 per cent was found by Fraps and Meinke (1945) who worked on the availability of carotene in different vegetables.

It is of interest to consider possible causes of such differences. It is almost certain that carotene in plants is present in several chemical forms, some of which are more readily absorbed by the animal than others. Aside from the different composition of carotene, the presence of isomers of beta-carotene can also be considered as a factor for the different degrees of availability in vegetables. The presence of these isomers neo-beta-carotene B and U has been demonstrated by Beadle and Zcheile (1942) and by Kemmerer and Fraps (1945) in certain fresh vegetables. These pigments are inseparable from beta-carotene when absorbed on magnesium oxide and therefore are included with the veta-carotene fraction when analyzed.

In the study of Kemmerer and Fraps (1945) it has been shown that neo-beta-carotene B possesses about $\frac{1}{2}$ and neo-beta-carotene U only $\frac{1}{4}$ to $\frac{1}{3}$ of the vitamin A activity of beta-carotene. Since most vegetables contain these isomers, their presence in the beta-carotene fraction in any considerable amount would significantly alter the potential vitamin A value.

Several investigators [Kemmerer and Fraps (1945) and Kelley *et al* (1950)] have shown that large amounts of xanthopylls (lutein) and chlorophyll when fed together with beta-carotene decrease the amount of utilization of the preformed vitamin A. Since lutein and other similar carotenoids are of common occurrence in natural foodstuffs, it is possible that the presence of these carotenoids may partly account for the low utilization of carotene in the vegetables studied.

When the total beta-carotene content and the corresponding per cent availability (Table 2) are taken into account, it is found that the sample of *mustasa* supplied 12.69 mg/100 g; *gabi* leaves, 10.51 mg/100 g; *saluyot,* 4.15 mg/100 g; and *kalabasa* tops, 2.49 mg/100 g of available carotene.

Although caution should be exercised in the interpretation of data obtained from rat studies in terms of human, it seems sound to speculate that vitamin A deficiency problem as found in nutrition surveys[5] conducted in various regions of the country might be attributed to the fact that apart from the low intake of vitamin A, a great deal of dietary vitamin A comes from plant food sources with poorly available carotene. If it can be assumed that poor absorption in rat means poor absorption in human, the results emphasize that when planning dietaries, carotene availabilities should be taken into consideration.

SUMMARY

The carotene availabilities of four vegetables were estimated using liver storage test with albino rats as experimental animals.

Relative to beta-carotene in oil, the per cent carotene availabilities of the four vegetables studied are as follows: *mustasa* 53.00 per cent; *gabi* leaves, 38.70 per cent; *saluyot,* 21.43 per cent; and *kalabasa* tops, 18.82 per cent.

The vegetables tested differed significantly from each other in carotene utilization, except *kalabasa* tops and *saluyot,* the difference of which was found not statistically significant.

Likewise, differences in the percentage carotene utilization of the control rats and those fed the different vegetables under test were found to be statistically significant.

When availability and total beta-carotene content were considered, it was found that the sample of *mustasa* supplied 12.69 mg/100 g; *gabi* leaves, 10.51 mg/100 g; *saluyot,* 4.15 mg/100 g; and *kalabasa* tops, 2.49 mg/100 g of available carotene.

The present data for four vegetables when compared with those obtained for four other vegetables in a similar experiment previously reported showed statistically significant differences in the carotene

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[5] E. S. Quioque *et al., loc. cit.*

utilization of the majority of the vegetables except in the case of *kamote* tops compared to *gabi* leaves and *kangkong* compared to *mustasa.*

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ON PHILIPPINE MOSQUITOES, VI. TRIPTEROIDES (TRIPTEROIDES) REISENI, A NEW SPECIES (DIPTERA: CULICIDAE)

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ONE TEXT FIGURE

ABSTRACT

Tripteroids (Tripteroides) reiseni, a new species of mosquito from Mt. Makiling, Luzon, is described and the male terminalia illustrated.

The new species of *Tripteroides* described herein was included in a recent listing of the Philippine Culicidæ (Basio, 1971), although no description nor taxonomic discussions were made. By external characters alone, the new species can not be separated from other allied species of Philippine *Tripteroides;* however, the differences are quite distinct in the male terminalia.

The classification follows that of Belkin (1962) who recognized three subgenera in the genus *Tripteroides*, namely: *Tripteroides* for the ornamented species, *Rachisoura* and *Rachionotomyia* for the non-ornamented species.

TRIPTEROIDES (TRIPTEROIDES) REISENI sp. nov.

Male: *Head.*—Vertex with distinct blue scale; silvery scales present laterally. Occiput with a mixture of brownish and silver scales. Torus and clypeus bare, dark-brown. Palpi light-brown, about 1.5 times length of proboscis. Proboscis slightly dark, about 1.5 times length of fore femur; silvery bristles present on underside medially.

Thorax. — Scutal integument dark brown, anterior and posterior areas darker; scutal scales narrow, dark brown; dorso-central bristles present. Scutellum with a mixture of yellowish to silvery broad and flat scales. Postnotum bare, brownish. Anterior pronotum silvery, with broad brownish scales and about 14 to 15 yellowish bristles. Posterior pronotum silvery with pale brown scales, with a single distinct dark bristle. Propleuron pale brown with 2 yellowish

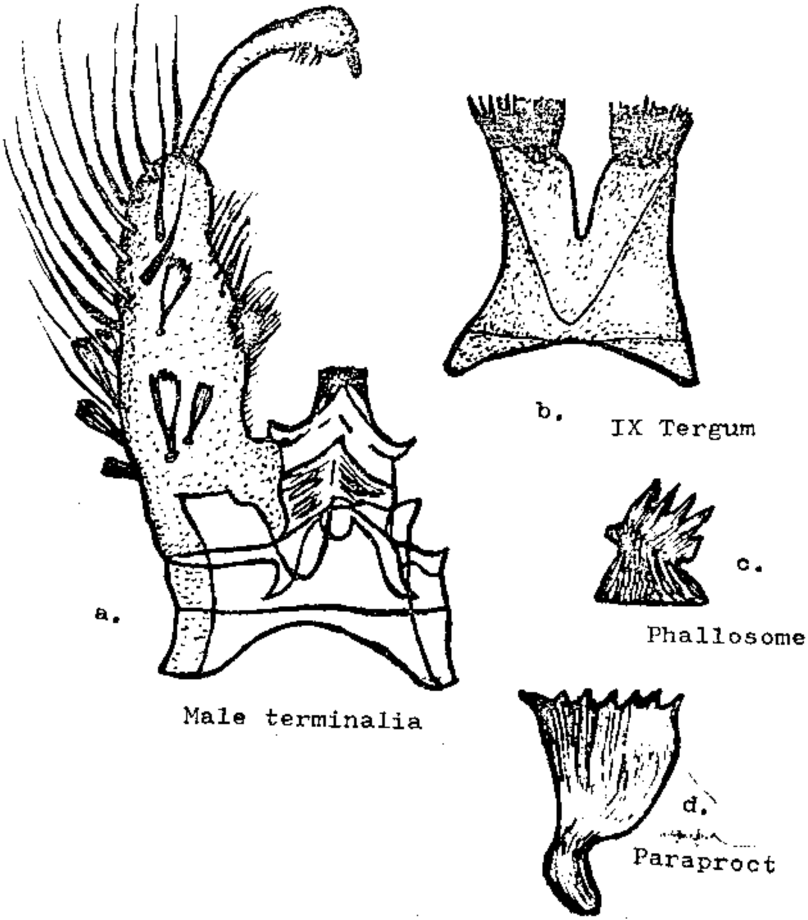

FIG. 1. *Tripteroides (Tripteroides) reiseni* sp. nov. Parts of male terminalia: *a*, Terminalia; *b*, ninth tergum; *c*, phallosome; *d*, paraproct.

stiff bristles. Pleuron pale brown. Sternopleural, mesepimeral, meron, subspiracular and postspiracular areas pale brown, silvery scales present on mesepimeron. Legs — silvery broad scales present on coxæ. Two silvery spots present on each femur, with yellowish scales on the anterior surfaces. Fore tarsal claws not equal; mid and hind tarsal claws equal and simple. *Abdomen* — Abdominal tergites all dark-scaled dorsally, with distinct patches of white scales on II to VII, not forming complete bands; tergites I and VIII dark-scaled. Sternites clothed with golden scales.

Terminalia — (Fig. 1). Basistyle with 7 to 10 stiff long bristles on the lateral sides; basal lobe with 7 stiff bristles and about 14 short bristles. Dististyle incurved a little swollen apically, with 6 short hairs toward apex. Apical flattened spiniform appendage short and dark. Ædeagus tapered with 3 to 8 teeth. Paraproct with 4 to 7 teeth and 2 to 6 cercal setæ. Lobes of tergite IX medially broad and triangular apically; each lobe bearing about 16 stiff and long apical bristles, almost equal in size and length.

Female, pupa and larva. — Unknown.

Type data. — Holotype male, collected on Mt. Makiling (elevation about 1,400 feet), Los Baños, Laguna Province, Luzon Island, Philippines, by R. G. Basio, January 13, 1969; with mounted terminalia, larval and pupal skins. Paratypes (all males) with mounted terminalia, pupal and larval skins (with same data as holotype). All types deposited in the Philippine National Museum, Manila.

Breeding habitat. — Arboreal pitcher plants (*Nepenthes* spp.)

Distribution. — Known only from the type locality.

Remarks. — This mosquito species is named after Capt. William K. Reisen, USAF, former chief, Entomology Division, Hq. 1st Medical Service Wing (PACAF), who served for sometime as one of the principal collectors of Philippine aquatic insects and mosquitoes. Having worked with him for a number of years, I express deep gratitude and appreciation.

PROXIMATE CHEMICAL COMPOSITION OF SOME COMMERCIAL GRADES OF ABACA (MUSA TEXTILIS NEE) FIBERS

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ABSTRACT

Seventeen commercial grades of abaca (*Musa textilis* Nee) fibers, ranging from the highest, AB, to the lowest, TOW, were analyzed. Based on the chemical composition of the fibers, the high average holocellulose (85.8 per cent), alphacellulose (60.8 per cent), and pentosan (16.3 per cent) contents and the low average ligning (10.0 per cent), ash (1.1 per cent), and silica (0.1 per cent) contents, and solubles in alcohol-benzene (1.2 per cent), caustic-soda (19.3 per cent), and hot water (1.9 per cent), make these fibers highly suitable for pulp and paper manufacture.

INTRODUCTION

Abaca fibers are claimed by Umali and Brewbaker (1956) to be the world's best natural rope-making material, especially for marine cordage which requires resistance to salt-water decomposition. The entry of synthetic fibers in the cordage field has gradually reduced the demand for abaca fibers. In view of this, other uses for abaca, preferably large-scale, should be investigated so as to save the industry.

Montgomery (1954) gave a brief description of the abaca (*Musa textilis* Nee) plant. It grows to a height of 4.5 to 7.5 m. Its true stem is fleshy, fiberless and quite small, about 5 cm or less in diameter. Leaf sheaths surround and support the true stem forming the pseudostem or "stalk" or "trunk" of the plant. Each leaf sheath has three layers. The outermost layer contains most of the fiber. The middle layer, of smaller intercellular spaces containing air, has a small amount of fine white fiber with lower tensile strength than those from the outer layer. The inner layer has no fiber.

Two basic operations are involved in the most widely used method of removing the fiber from the leaf sheaths: (*a*) "tuxying" or separating the fibrous outer layer or "tuxy" from each leaf sheath and (*b*) "stripping" or removing the pulpy material from the "tuxy" to obtain the clean fibers.

Both operations must be performed immediately after felling the stalk. The stripping operation is accomplished by hand stripping or spindle stripping to free the fibers from the pulpy material remaining on its surface. Decorticating is employed to free abaca fibers from whole leaf sheaths or stalks.

There used to be 16 normal grades, 10 residual grades, and one waste grade in the classification of hand-stripped abaca fibers by Lim (1961). These were reduced to 10 normal grades and four residual grades; the waste grade was replaced with the grade "Wide strips" or WS in the later revision by Balatbat (1967). Lim (1961) states that the term "fiber" refers to its common and commercial significance, not to its scientific meaning. The "fibers," according to Montgomery (1954), are actually bundles or aggregates of individual cells or fibers as dealt with in papermaking.

Grades are determined according to strength, extent of cleaning and color. In addition, the texture and length are often necessary to identify type in any of the grades. The normal tensile strength varies with the extent of cleaning as well as the source of the fiber. Theoretically, the cleaner the fiber, the higher is the strength. Fibers from the outer sheaths have generally higher tensile strength than those from the inner sheaths.

The four recognized classes or groups of cleaning for hand-cleaned abaca are: excellent, good, fair, and coarse. Cleaning determines the group of grades to which any fiber belongs, while color determines the grade. The nearer the sheaths are to the outer portion of the abaca "trunk," the darker is the color of the fibers; hence, the lower the corresponding grade.

The residual grades are assigned for damaged abaca fibers for the purpose of clearing any normal grades of any damaged fiber that would decrease its tensile strength which is the most important property of the fibers responsible for the high reputation of abaca. "Waste" or "Wide strips" are abaca fibers of very irregular cleaning, too strippy and woody to fit in any of the normal and residual grades.

One of the best outlets for the abaca fibers is the pulp and paper industry. In fact, abaca fibers, in the form of old ropes, have been used abroad in the production of high strength bag and other quality papers. Other possible uses for abaca would be for the manufacture of dissolving pulp, the raw material for viscose rayon and other cellulose derivatives.

To fully evaluate not only the pulping qualities but also other potential uses of a fibrous raw material, its chemical composition has to be determined.

This report presents the chemical analyses of 17 commercial grades of hand-stripped abaca fibers for general cordage purposes. These fibers were graded in accordance with the classification standards of the Bureau of Fiber Inspection Service as given by Lim (1961).

EXPERIMENTAL

Raw material preparation. — The Abaca and Other Fibers Development Board and the University of the Philippines, College of Agriculture furnished the abaca fibers used in this study. The materials, as received, were in the form of long fiber strands sufficiently air-dried for processing. The fibers were cut manually to about 1.0 to 1.5 cm long, then ground in a Wiley mill. The portion of each sample, passing through a 40-mesh screen and retained on a 60-mesh screen, was used for analysis.

Chemical analyses. — The appropriate TAPPI (1970) methods were used to analyze for ash, lignin, pentosans, and solubilities in alcohol-benzene, caustic soda and hot water. Holocellulose was calculated by difference (per cent holocellulose = 100 — per cent alcohol-benzene extracts — per cent hot water solubles — per cent lignin — per cent ash). Alpha cellulose was analyzed by the TAPPI method for pulp after a preliminary chlorite treatment by the method of Wise *et al* (1945). Silica was determined by a method developed by Nicolas (1967).

RESULTS AND DISCUSSION

The results of the analyses are given in Table 1. Two replicate analyses were made for each fiber grade. The grade differences were significant for all the components analyzed as determined by an analysis of variance. In general, group averages for holocellulose and alpha-cellulose increased; those for lignin, pentosans, ash, and alcohol-benzene, hot water and 1-per cent NaOH solubles decreased as the degree of cleaning (for the normal grades) improved.

Except for 1-per cent NaOH solubles, the chemical components of the residual grades were within the ranges of those of the normal grades, since these residual grades consisted of fibers rejected from the normal grades due to discoloration, low tensile strength,

TABLE 1.—*Proximate chemical composition in per cent of some commercial grades of abaca fibers (Moisture-free basis).*

Grade	Alpha-cellulose	Holo-cellulose	Lignin	Pentosans	Solubles in			Ash	Silica
					Alcohol-benzene	Hot water	1 per cent NaOH		
Excellent cleaning (Grades AB, CD, E, S_2, S_3)	64.3	88.3	8.8	15.9	0.9	1.4	17.8	0.6	0.04
Good cleaning (Grades I, J1, G.H)	59.6	86.4	10.2	16.3	1.2	1.4	18.6	0.9	0.06
Fair cleaning (Grades J2, K, M1)	58.3	84.8	10.6	16.3	1.2	2.2	19.1	1.2	0.13
Coarse cleaning (Grades L1, L2, M2)	58.7	82.8	10.8	16.5	1.5	3.1	21.3	1.8	0.21
Residual (Grades Y3, TOW)	61.4	84.6	10.4	17.1	1.2	2.2	21.8	1.5	0.14
Mean of all grades	60.8	85.8	10.0	16.3	1.2	1.9	19.3	1.1	0.10
Range	56.8-69.7	80.6-89.2	7.6-11.6	14.8-18.2	0.3-1.8	0.3-4.2	13.4-25.0	0.2-2.3	0.01-0.30

short length (less than 60 cm), and other factors otherwise affecting the quality, i.e., the color, uniformity, and strength of the normal grades.

On the other hand, the grades within the groups did not exhibit a consistent trend for the different components despite the determination of grading by color which, in turn, depended primarily upon the position within the stalk of the sheaths producing the fibers. No trend was discernible even in the excellent-cleaning group which contained the cleanest fibers. In the less-cleaned groups, other factors affected the color such as careless stripping and handling of the fibers. These could reduce the classification of fibers by one or more full grade following Balatbat's (1967) and Lim's (1961) recommendations.

Ash represents the noncombustible, nonvolatile and inorganic portion of the raw material. A high ash content in the fiber is objectionable, especially if carbonates are present and the fiber is intended for the manufacture of dissolving pulp. Furthermore, Grant (1958) states that the presence of iron, copper, or manganese may produce discolored pulp. Silica is objectionable in the chemical recovery system of pulp mills.

The ash content ranged from the lowest value of 0.2 per cent for Grade E to a maximum of 2.3 per cent for Grade L1, with an average value of 1.1 per cent. The silica content was lowest for Grade E, 0.01 per cent, and highest for Grade L1, 0.30 per cent. With silica contents lower than those of bamboos successfully used in India, where Bhargava's analyses (1946) of various species averaged 1.3 per cent, it may be safe to assume that these raw materials would present no silica problem in the pulp mill.

Lignin is an amorphous polymeric product which, as the cementing substance, gives rigidity to the cell walls of the fibers. From a pulp manufacturer's point of view, lignin is considered a nuisance; hence, materials with high lignin contents are less desirable for pulp and papermaking.

Grade CD fiber gave the lowest lignin content of 7.6 per cent; the highest, 11.6 per cent for Grade J1. The average lignin content was 10.0 per cent for all grades.

Extractives are the components in ligno-cellulosic materials which can be removed by such solvents as alcohol-benzene and hot water. Fibrous materials with high extractives are generally undesirable for pulping as they not only give lower pulp yields but consume more pulping chemicals.

The alcohol-benzene solubility is recommended by TAPPI (1970) as a measure of waxes, fats, resins, and some ether-insoluble or water-soluble components. The lowest alcohol-benzene extract of 0.3 per cent was obtained from Grade E; the highest, 1.8 per cent for Grade L1. The average value for all grades was 1.2 per cent.

Hot water solubles may include organic salts, sugars, cyclitols, gums, pectinlike materials, galactans, tannins and pigments. The hot water solubles obtained ranged from a low 0.3 per cent for Grade J1 to 4.2 per cent for Grade L1 and averaged 1.9 per cent for all the grades analyzed.

The solubility in 1-per cent caustic soda determines the resistance of the sample to solution by hot dilute alkali. It also indicates the degree of fungal decay in a fibrous material. As a fibrous material decays, the caustic solubility increases and, consequently, results in a corresponding decrease in pulp yield. The percentages of alkali-soluble components ranged from 15.4 per cent for Grade S3 to 25.0 per cent for Grade L1. The mean for all the grades analyzed was 19.3 per cent.

The holocellulose includes the cellulose and the hemicelluloses of a fibrous material. A material with high holocelluose content woud be desirable for pulping. Grades E and S3 gave the highest holocellulose content of 89.2 per cent and the lowest, 80.6 per cent for L1. The average value obtained for all grades was 85.8 per cent.

The alpha-cellulose is that part of the holocellulose which is resistant to a 17.5-per cent solution of NaOH. It consists of cellulose molecules with high but varying molecular weight. The alpha-cellulose ranged from 36.8 per cent for Grade 1 to 69.7 per cent for Grade E. The average alpha-cellulose of the various grade was 60.8 per cent.

Pentosans are part of the noncellulosic carbohydrates or hemicellulose present in a fibrous material. Pulps with high pentosan content require shorter beating to a certain freeness level than those with low pentosan content. Furthermore, the Food and Agriculture Organization reports that the higher the pentosan content, the denser is the pulp sheet with correspondingly higher burst, tensile strength and folding endurance. Grade J1 was the lowest in pentosan content with 14.8 per cent; Grade G, the highest with 18.2 per cent. The average pentosan content of the various grades was 16.3 per cent.

Compared with the average for a group of *Agave* and *Furcraea* fibers studied by Escolano *et al* (1970), these abaca fibers had somewhat lower average holocellulose (- 2.8 per cent), alpha cellulose (- 4.8 per cent), alcohol-benzene solubles (- 0.5 per cent), and pentosan (- 2.6 per cent), higher lignin (+ 3.0 per cent), hot water solubles (+ 0.3 per cent), caustic soda solubles (+ 3.4 per cent) and similar ash. Compared with the results of Semana *et al* (1968, 1970) on Philippine hardwoods and bamboos and those of Francia *et al* (1970) on softwoods, these abaca fibers had much higher holocellulose, much lower lignin, alcohol-benzene and hot water solubles, lower ash than the bamboos and similar pentosans and caustic soda solubles to those of the hardwoods. These fibers had lower silica content than the *Dendrocalamus strictus* bamboo, the major species reported by Bhargava (1945) as successfully being used by the Indian sulfate pulp mills.

The experimental results indicate that even the most inferior of the normal grades, M2, or even the residual grades such as Y3 and TOW, are still suitable raw materials for pulp and paper.

CONCLUSION

The degree of cleaning of the abaca fibers greatly affected the proportion of their components. The better the cleaning, the higher were the holocellulose and alpha-cellulose contents and the lower were the pentosans, the alcohol-benzene, hot water and 1-per cent NaOH solubles, ash, and to a lesser degree, the lignin. The effect of grading within the groups was apparently less pronounced than the effect of degree of cleaning on the chemical composition.

Based on the results of the chemical analysis, the various grades of abaca fibers studied are highly desirable for pulp production, both for paper or dissolving (viscose) grades. All the attributes of a good pulping material, such as high holocellulose, alpha-cellulose, and pentosan contents, and low lignin, extractives and ash, are present in the samples tested.

A possible disadvantage is the larger digester capacity required per ton of pulp produced due to the bulky nature of the abaca fibers compared with wood chips. Considering the low lignin and extractive contents, these abaca fibers would require milder pulping conditions compared with the same quantity of the traditional pulp and paper raw materials such as softwoods, bamboos and hardwoods. This involves lower pulping chemical consumption and/or shorter pulping time and, therefore, smaller chemical recovery plants and lesser steam requirements. The higher the holocellulose content, the higher is the pulp yield per ton of the raw material.

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ELECTRONIC AND STRUCTURAL EFFECTS ON RATES AND EQUILIBRIA, II

LINEAR FREE ENERGY RELATIONSHIP IN POLAROGRAPHIC REDUCTION OF NITROARENES *

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SEVEN TEXT FIGURES

ABSTRACT

Twelve substituted nitrobenzenes (nitroarenes) and benzaldehyde were polarographically reduced using the dropping mercury electrode method. The half-wave potentials which are dependent on the electronic and structural effects inherent in the compounds under study were obtained for the *ortho*-, *meta*-, and *para*- substituted nitroarenes. Linear free energy correlation was observed with the *meta* and *para* isomers but the *ortho* analogs deviated significantly and could be correlated among themselves. Results are explained in terms of *ortho* or proximity effects and other modern chemical theories.

Modern organic chemistry emphasizes the fluid integration of structure and reactivity [Hendrickson *et al* (1970), Hine (1962), Lim-Sylianco (1971), March (1968)] as can be observed in any contemporary international journal and in current textbooks. Even with this trend, there are still many other families of organic compounds left uncorrelated. Of all nitrogen containing compounds, the nitrocompounds have been the least studied as far as the extrathermodynamic approach or better known as the Hammett free energy correlation, is concerned. And for this reason we undertook this polarographic study.

It should be noted also that electron transfer reaction involving nitrogen oxides in nitrogen fixation [Conn and Stumpf (1966)] occurring in nature might have some relationship with this type of organic system.

The nitrogen in the nitro group (-NO_2) possesses a formal positive charge and because of this significant feature, it has been known as one of the most electroactive functions in organic chemistry

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* For previous paper in this series, see W. Y. Lim and S. de Vera "Polar" and Steric Effects on Rates and Equilibria. I. Nucleophilicity of Some Aromatic Amines," Natural and Applied Science Bulletin (NSDB, University of the Philippines **22** (1970). 26-37.

[1] Part of the M. S. dissertation of Mr. Ku was included in this paper.

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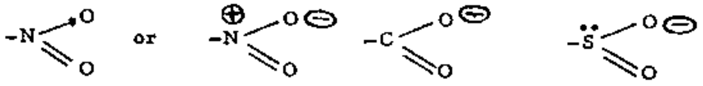
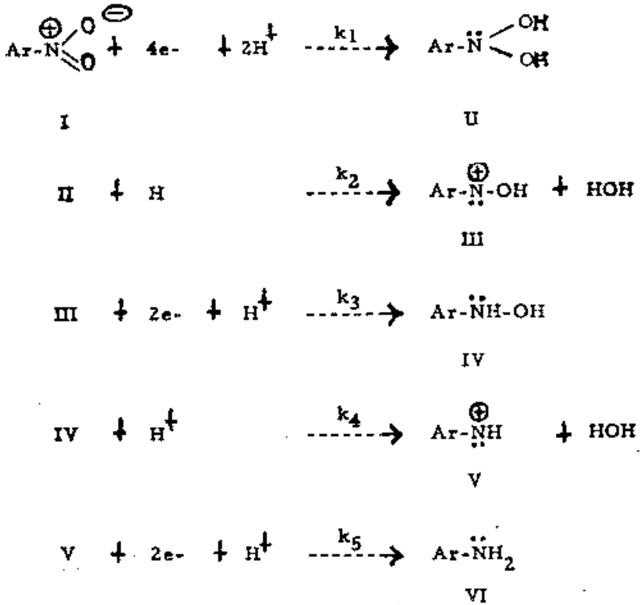
[Zuman (1969)]. Polarography is by far more convenient and accurate than other chemical methods [Hickenbottom (1957)] for the study of the nature of nitrocompounds. There are other isoelectronic groups, such as the carboxylate anion and the sulfinate anion, but these do not possess a formal positive charge at the central atom.



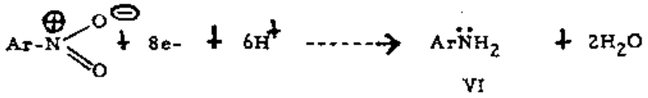
nitro group carboxylate group sulfinate group

The mechanism of polarographic reduction of the nitro group has been proposed by many authors [Perrin (1965), Zuman (1967)]. This can be easily summarized in the following scheme:

SCHEME I

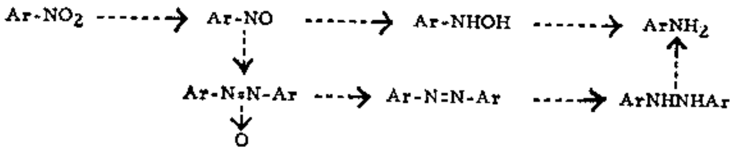


The overall reaction then would be:



This electrolytic reduction, however, has also been proposed differently by Haber through a separate scheme (see Scheme II). In his scheme he proposed the intervention of azoxy intermediate, VII, the azo compound, VIII, which eventually would be reduced to the hydrazines, IX, and finally to the amine [Stocescova (1949)].

SCHEME II



VII VIII IX

The dihydroxylamine intermediate, II, in Scheme I is quite reactive since it has similar electronic characteristics as the geminal diols [Fuson (1962)] which undergo dehydration with ease. This effect has been explained to be due to competitive induction coming from two electronegative oxygen atoms bound to the same carbon center. In aqueous medium this intermediate II can easily undergo fast protonation and dehydration. The formation of hydroxylamine III could either come from this process in Scheme I or through the nitrosocompound in Scheme II. Both the nitrosocompounds and the hydroxylamines are isolable. It was further noted by Haber that nitrosocompounds have not been detected in polarography since the reduction potential of nitrosoarenes is more positive than nitroarenes. These schemes are proposed on the basis of the past findings and the most acceptable intermediates. Under basic and neutral conditions, the hydroxylamine, IV, was isolated as the end product of polarographic reduction [Kolthoff and Lingane (1962)] while under acidic conditions, the hydroxylamine was observed to proceed to the amine, VI. There are only two observable half-wave potentials for the nitro group [Stocescova (1949)] and this could be taken to mean that step 3 (Scheme I) takes place very fast, hence it is not discernible as compared with steps 1 and 5 of Scheme I.

On consideration of these observations and other pertinent information, the polarographic reduction of nitroarenes and benzaldehyde was conducted. Half-wave potentials were chosen instead of the limiting current since the former is independent of concentration [Hendrickson *at al* (1970)] and is only affected by electronic and structural influence within the molecule.

EXPERIMENTAL PART

All nitroarenes except for *m*-chloronitrobenzene and *p*-nitroaniline obtained from British Drug House, Ltd., and *o*-bromonitrobenzen, *o*-nitrobenzaldehyde and benzaldehyde from Merck, were procured from Eastman Kodak Company. Nitrobenzene was obtained from J. T. Baker Chemical Company. Using standard procedures, all chemicals were purified before use.

All melting point determinations were taken from an Hoover Capillary Melting Point Apparatus. All pH determinations were taken from a beckman Zeromatic SS-3 pH Meter. An E. H. Sargent Model XXI Polarograph was used in all polarographic measurements. The substrates were separately prepared at a concentration of 10^{-3} M in 50-per cent alcohol buffer (phosphate) at pH 7.00. Each one was deaerated with purified nitrogen gas for 3 minutes before running in the polarograph using an H-cell, 0.03-per cent gelatin suppressor and a drop time of 3 seconds. All measurements were made at 24 to 25°C at a voltage range of 0 to 2 volts and a sensitivity setting of 0.400. Half-wave potentials were calculated following the standard procedure [Manual for Sargent Model XXI Polarograph (1958)].

RESULTS AND DISCUSSION

Half-wave potentials of model nitroarenes (Table 1) were obtained from the polarograms (Figures 1-6) using established procedure [Manual for Sargent Model XXI Polarograph (1958)]. It is evident from Table 1 that the trend of reduction for nitroarenes (towards less negative half-wave potential or greater ease of reduction) is with increasing electron attracting (electron defficient) substituents. This is observed with the *meta*- and *para*- substituted nitroarenes. The *ortho*- substituted nitroarenes show incoherent values. Considering all the *para*- substituted nitroarenes, the trend observed was as follows:

Half-wave potentials of some nitroarenes and benzaldehyde at 10^{-3} M vs. S.C.E.

Compound	—E 1/2, volt
benzaldehyde	1.467
p-nitroaniline	0.886
p-nitrophenol	0.847
p-nitrotoluene	0.743

m-nitroaniline	0.734
nitrobenzene	0.731
p-nitrobenzoic acid	0.723
o-nitrophenol	0.665
o-chloronitrobenzene	0.662
p-chloronitrobenzene	0.651
o-bromonitrobenzene	0.638
m-chloronitrobenzene	0.618
o-iodonitrobenzene#	0.605
o-nitrobenzaldehyde	0.522, 0.763, 1.516
p-idonitrobenzene#	0.510

#values obtained from Colichman and Liu (1954).

*Based on average of these trials. All values have on average error of ± 0.002v.

$$p\text{-I} > p\text{-Cl} > p\text{-COOH} > p\text{-H} > p\text{-Me} > p\text{-OH} > p\text{-NH}_2$$

The substituent constants [Ehrenson (1964), Hammett (1970), Hine (1962), Jaffe (1953), Ricthie and Sager (1964)] as obtained from similar aromatic systems support the above propensity (Table 2). The source

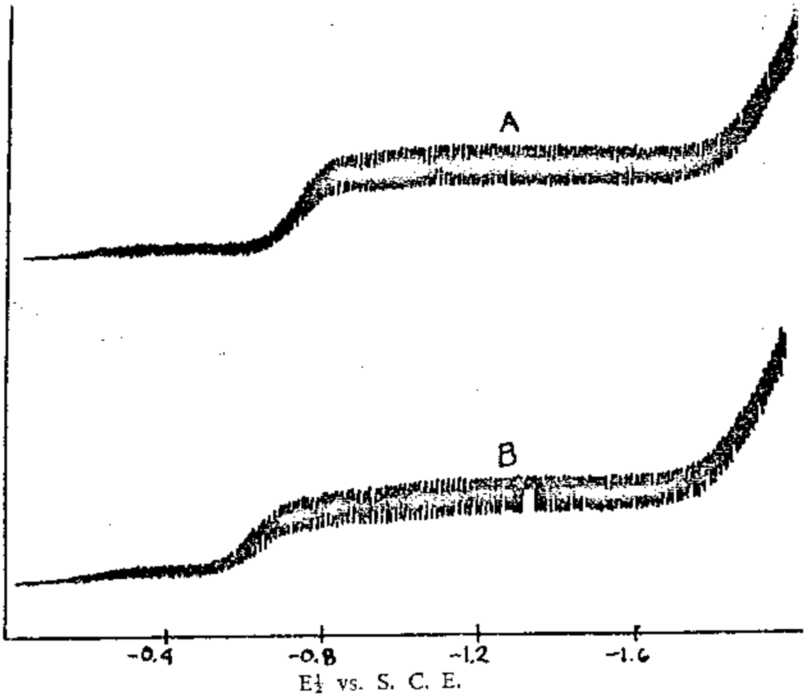


FIG. 1. 10^{-3}M nitrobenzenes in 50-per cent alcohol buffer, pH 7.00: *A*, notrobenzene: *B*, o-bromonitrobenzene.

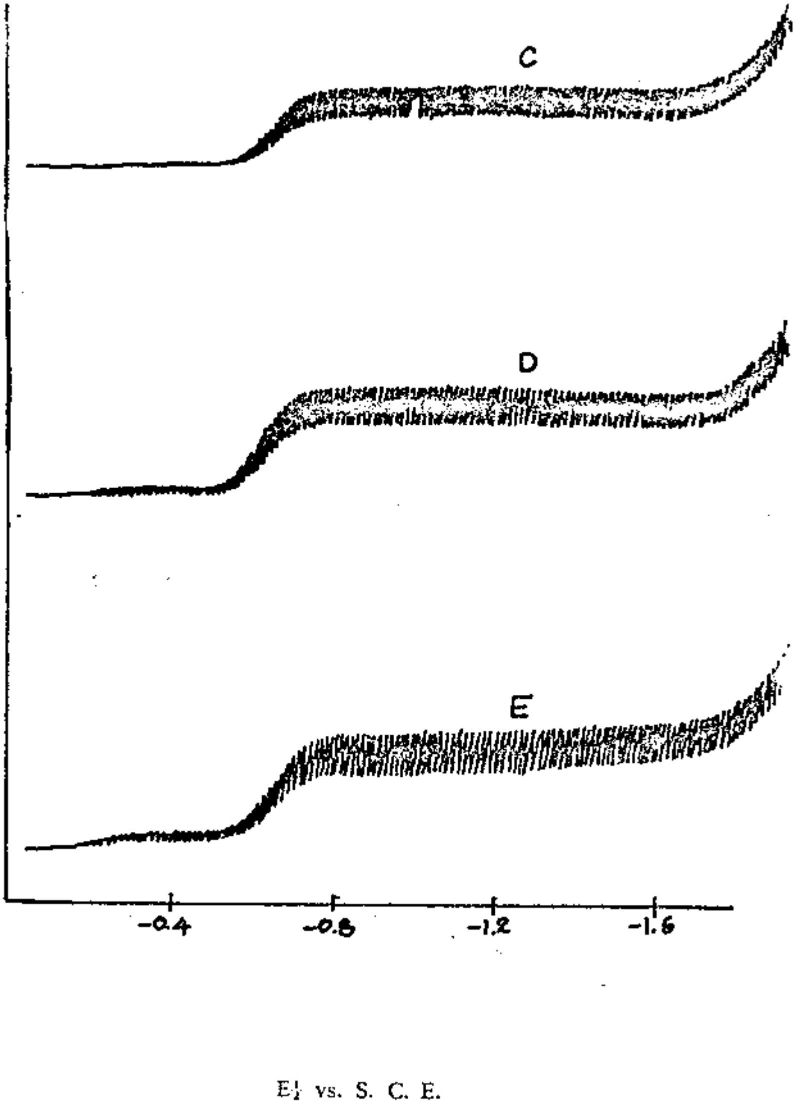


FIG. 2. 10^{3}M nitrobenzenes in 50-per cent alcohol buffer, pH 7.00: *C*. o-chloronitrobenzene; *D*. m-chloronitrobenzene; *E*. p-chloronitrobenzene.

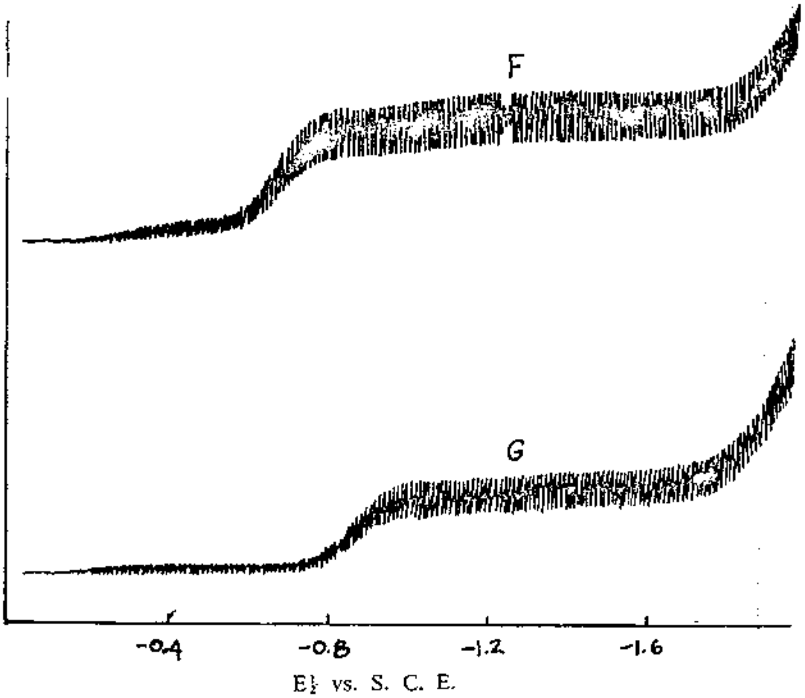


FIG. 3. $10^{-3}M$ nitrobenzenes in 50-per cent alcohol buffer, pH 7.00: *F*, o-nitrophenol; *G*, p-nitrophenol.

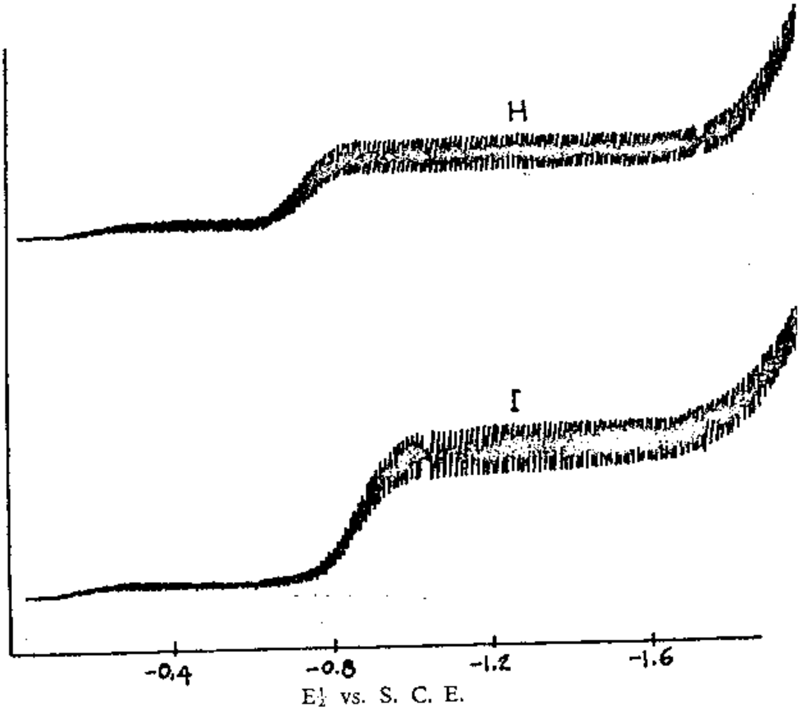


FIG. 4. $10^{-3}M$ nitrobenzenes in 50-per cent alcohol buffer, pH 7.00: *H*, m-nitroaniline; *I*, p-nitroaniline.

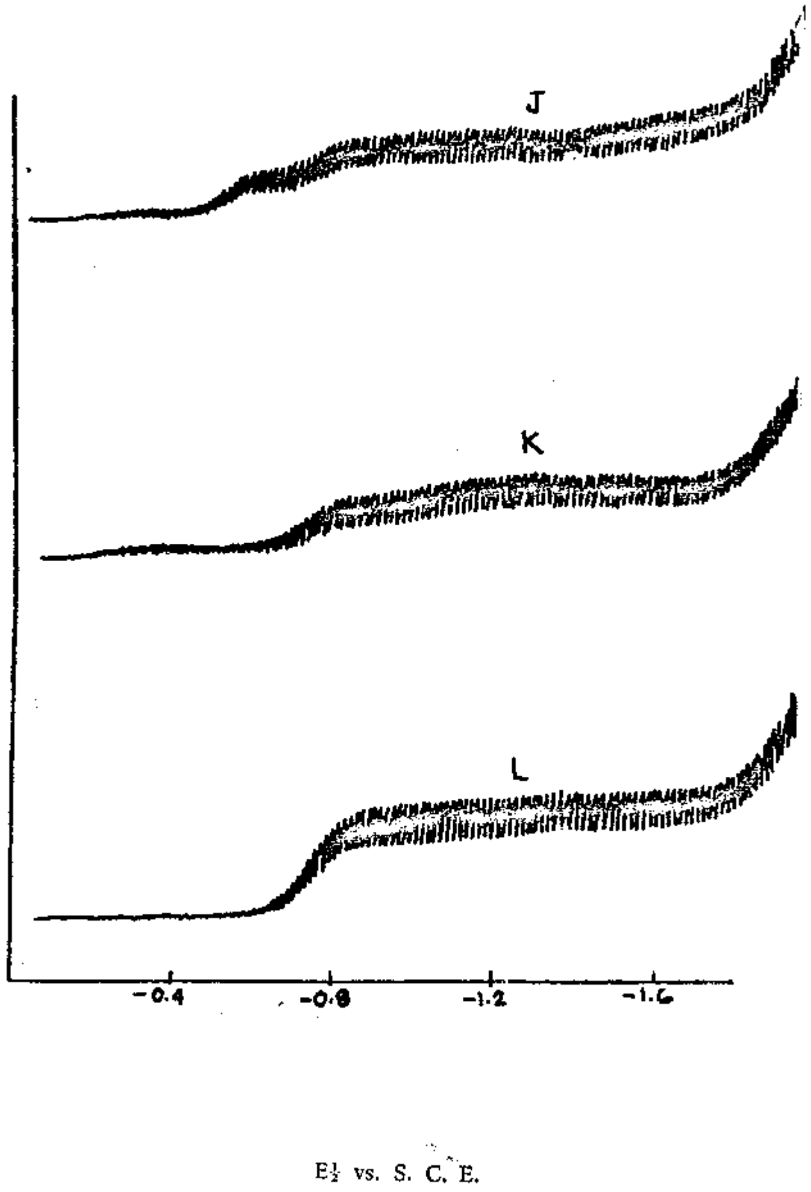


FIG. 5. 10^{-3}M nitrobenzenes in 50-per cent alcohol buffer, pH 7.00: *J*, o-nitrobenzaldehyde; *K*, p-nitrobenzoic acid; *L*, p-nitrotoluene.

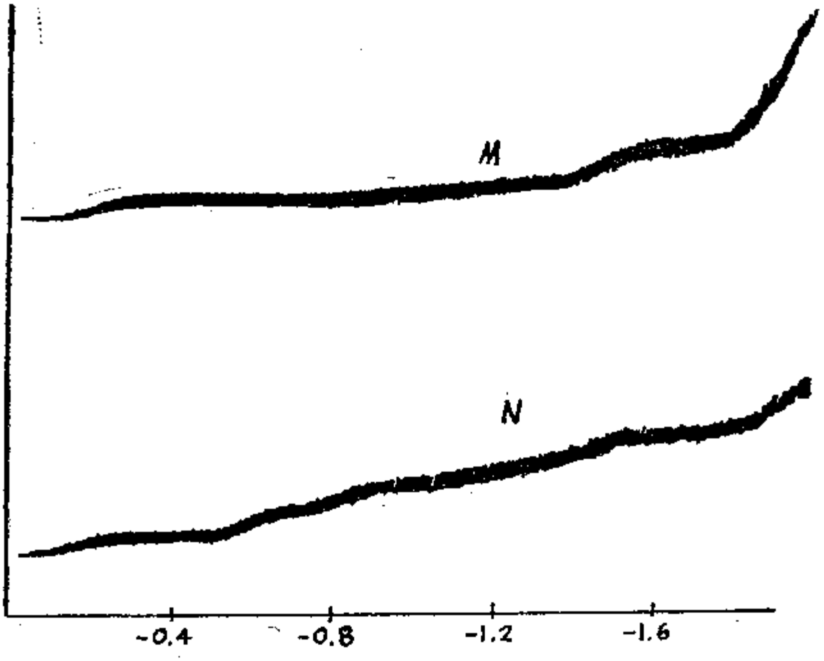


FIG. 6. 10^{-3}M nitrobenzene and benzaldehyde 50-per cent alcohol buffer. pH 7.00: *M*, benzaldehyde, *N*-o-nitrobenzaldehyde with gelatin at 5 x 10^{-5} per cent.

of these constants was benzoic acid and its derivatives. The carboxylate anion is isoelectronic with the nitro group, hence the choice of using

TABLE 2.—*Hammett substituent constants.*

Substituent	σ_p (10,22)	σ_m (24)	σ_o (18)	σ_I (18)
H	0.00	0.00	0.00	0.00
CH_3-(Me)	-0.17	-0.07	-	-0.05
COO^-	0.00	0.10	-	-
OH	-0.37	0.09	1.22	0.25
NH_2	-0.66	-0.04	-	0.10
Br	0.23	0.39	1.35	0.45
Cl	0.23	0.37	1.28	0.47
I	0.18	0.35	1.34	0.39

benzoic acid derivatives is valid. The substituents at the *para* position exert both inductive and resonance effects. In cases where lone pairs of electrons are present, such as in the halogens, the hydroxy and amino groups, resonance effects in the opposite direction to that of inductive influence seem to outweigh electron attraction. In the halogens, as the size of the element increases, resonance contribution decreases, hence inductive influence slowly overcomes resonance effects. The bigger the size of the *p*- orbitals in which the lone pair of electrons resides, the lesser would be the degree of maximum overlap between the halogen orbital and the adjacent carbon orbital in the ring, thereby causing less effective resonance assistance [Hine 1962), Ricthie and Sager (1964)] of the substituent to the aromatic ring.

There were only two *meta*- substituted nitroarenes available for our study and the observed trend with nitrobenzene as the reference followed the predicted order shown in Table 2.

$$m\text{-}Cl > m\text{-}H > m\text{-}NH_2$$

The amino group at the *meta* position does not exert as much electron donations as is in the *para* position as shown from the half-wave potentials in Table 1 and the sigma constants in Table 2. The less negative the half-wave potential becomes, the greater the ease of reduction. The amino group at the *meta* position becomes more electron attracting than at the *para* due to the absence of direct resonance interaction of the lone pair of electrons on nitrogen with the reactive site (the nitro group). As a consequence this enhances the reduction of the nitro group which is now rendered more positive. It is interesting to note that the amino group and the chloro substituent differ in their sigma constants as well as their effects on the reduction of the nitro group when the electronegativity of nitrogen and chlorine is the same [Gould (1959), Hendrickson *et al.* (1970), Ingold (1970), Mahler and Cordes (1971)]. Again the feature due to size becomes a critical factor in this case. The difference in the half-wave potential of chloro and amino functions at the *meta* is only 0.116 v with a corresponding difference in sigma constant of 0.41, whereas at the *para* the difference is larger, 0.235 v with a corresponding gap in sigma constant of 0.89. These values support the previous statements regarding the opposing inductive and resonance influences operating in the model compounds under study.

Taking the *ortho* series into consideration, the trend observed is as follows:

$$o\text{-}CHO > o\text{-}I > o\text{-}Br > o\text{-}Cl > o\text{-}OH > o\text{-}H$$

Simple electronic and structural models would not suffice to explain the trend exhibited by the *ortho*- substituted compounds. The order shown above does not follow ordinary substituent or sigma constants [Charton (1969), Colichman and Liu (1954), Jaffe (1953), Ricthie and Sager (1964), van Bekkum *et al* (1959), Wells (1963)] and because of this, many authors [Jaffee (1953), van Bekkum *et al* (1959), Wells (1963)] have proposed separate substituent constants for the *ortho* substituents. This has added more confusion to the ever increasing sliding-scale of sigma constants in the literature [Hammett (1970)]. However, it is imperative that the *ortho* series should be studied and differentiated from the *meta* and *para* counterparts. It is very well known that *ortho* substituents [Newman (1956)] due to proximity to the reactive site exert steric influence in addition to induction and resonance effects. Steric effects could alter the reactivity in a less predictable manner because the nitro group is rendered noncoplanar with the aromatic ring thereby causing less interaction between the substituent and the reactive site. Another possible reason is the alteration of the symmetry of the nitro group due to steric effects and it is well known that symmetry plays a significant role in reactivity of organic molecules [Pullman and Pullman (1952), Volmer and Servis (1968), Woodward and Hoffmann (1970).

On the other hand, a more applied analysis has been put forth by Zuman (1960, 1969) and he has called this the "polarographic ortho shift." This ortho shift could either be negative or positive and has been defined as follows:

$$(E_{1/2})_{o-x} - (E_{1/2})_{p-y} = \Delta_o \text{ (ortho shift)}$$

where: $(E_{1/2})_{o-x}$ and $(E_{1/2})_{p-y}$ are half-wave potentials of the *ortho* and *para* substituted respectively.

If the difference shows a positive value, the reduction of the *ortho*

TABLE 3.—*Polarographic ortho shift.*

Substituent	Ortho shift
OH	0.182
Cl	-0.011
I	-0.095

isomer takes place with greater ease than the *para* isomer. As Shown in Table 3, and with consideration of what data have been reported in the literature on ortho shifts, the common trend

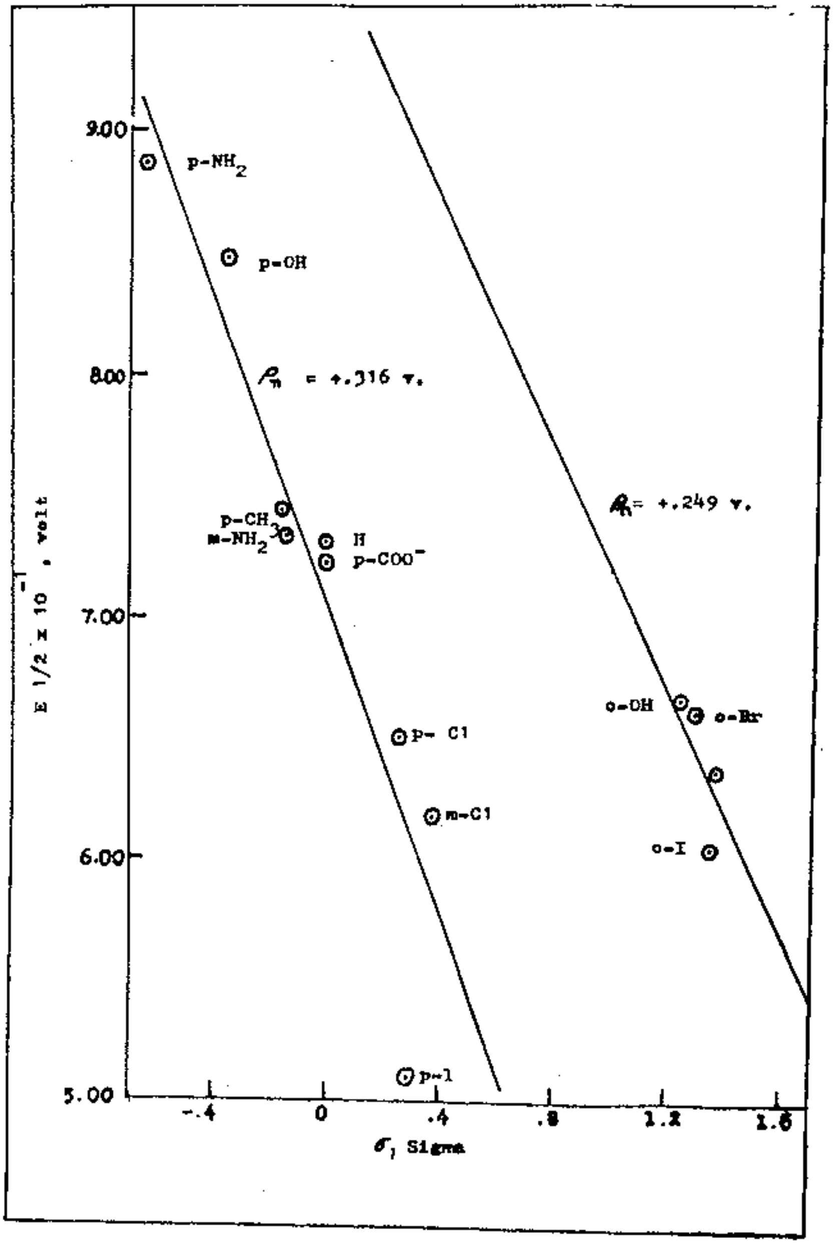
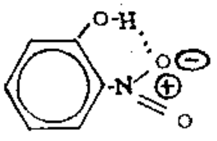
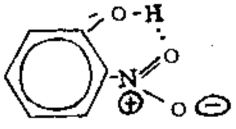


FIG. 7. Half-wave potentials of substituted nitrobenzenes in 50-per cent C_2H_5OH buffer vs. S.C.E. plotted against σ values.

is towards the negative value, meaning, that *ortho* substituents generally render the reactive site less reducible. In the case of *o*-nitrophenol, the value is positive. From examination of the Dreiding model of this compound. intramolecular hydrogen bonding is observed as shown by structures A and B. This intramolecular hydrogen bonding stabilizes the molecule and renders the nitro group closer to the electron attracting oxygen at the *ortho* position. This results in greater ease of reduction as compared with the *para* isomer (Table 1).


A


B

A linear free energy plot was done (Figure 7) employing all the nitroarenes is this study. It is observed that the *ortho* isomers do not fall on the same straight line (by method of least squares) as those of the *meta* and *para* analogs. The *ortho* compounds, however, could be correlated among themselves, indicating that steric effects make the *ortho* series abnormal as compared with the others. The slope of the line for the *ortho* series is smaller which explains the nature of diminished transmission of substituent effects to the reactive site [Newman (1956), Ricthie and Sager (1964)]. The smaller the slope the weaker would be the transmission capability of a given substituent.

Another unusual observation is on the *o*-nitrobenzaldehyde. Initially we wanted to see the effect of an electron attracting carbonyl function on the reduction of the nitro group. During the inquiry there were only two inflections observed on the polarogram for this compound and since the values checked with those in the literature [Kolthoff and Lingane (1962), Perrin (1965), Zuman (1967)] we disregarded the fact that the benzaldehyed group (or formyl group) could also be reduced. When we altered the concentration levels of our gelatin suppressor, a faint third inflection was observed at 1.516 volts. We were forced to check this value by employing benzaldehyde itself under the polarographic conditions used and indeed the parent aldehyde showed a half-wave potential at 1.467. The third inflection of *o*-nitrobenzaldehyde then is attributed to the formyl group. The other half-wave values of 0.522 and 0.763 volts are due to the reduction potentials of the nitro group and the subsequent hydoxylamine intermediate respectively. These findings refine further those obtained by kolthoff and Lingane (1962).

With this work we feel that we have provided more data to the insufficient information regarding polarographic values of organic nitro compounds and that we have observed interesting structure-reactivity patterns which could not have been possible by other means. As an extension we are pursuing this work to include other isomers of nitrophenol, nitroaniline, nitrobenzaldehyde and nitrobenzoic acid. A detailed screening on the effect of pH on the polarographic reduction of nitrobenzoic acids is also being conducted. Another colleague has been doing extensive work on the polarographic reduction of substituted benzaldehydes.

ACKNOWLEDGMENT

The authors wish to express their appreciation for the support extended by the Department of Chemistry and staff, to Dr. C. Y. Lim-Sylianco for the use of some of her equipment and laboratory space as well as her stimulating discussions, and to Dr. R. Montemayor for his criticisms.

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THE FROGS AND TOADS OF TJIBODAS NATIONAL PARK, MT. GEDE, JAVA, INDONESIA

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University of Queensland, Brisbane, Australia

TWO PLATES AND SEVEN TEXT FIGURES

ABSTRACT

Brief notes on adult frogs and toads with their habitats, altitudinal distribution and a key are presented for 19 species of 11 genera which occur in the Tjibodas National Park, Java. Bufonidæ is represented by two genera with four species, Pelobatidæ by two genera with two species, Microhylidæ by one genus with two species, Ranidæ by three genera with seven species, and Rhacophoridæ by three genera with four species.

The Tjibodas frogs and toads are neatly divided between the primary rainforest and nonprimary rainforest dwellers. There is a decrease in the number of species with the increase of altitude, nine species at 1,500 m, five species at 2,000 m, and only one species at 2,500 m above sea level. The vegetation appears to play a major role in the distribution of Javanese frogs and taods.

The Tjibodas National Park is about 15 sq km and is the most accessible and undisturbed tropical rainforest in Java. It ranges from 1,400 to 3,300 m above sea level on the southeastern slope of Mt. Gede, 40 km south of Bogor and about 6 km off its main Bogor to Bandung highway.

The Tjibodas Botanical Garden is only 0.5 sq km and lies adjacent to the southeastern border of the Tjibodas National Park. It contains native mountain and other subtropical plants.

There has been no comprehensive work on frogs and toads from the Tjibodas National Park. Unfortunately this study was terminated before life cycles could be determined. This paper contains brief notes on adult frogs and toads with their habitats and altitudinal distribution and a key to differentiate 19 species in that area. Brief field studies were conducted in 1961-1962, and more intensive surveys in August 1963, March and May 1964. The Tjibodas Botanical Garden guest house, and camps within the forest at Tjibeuereum waterfall (1,700 m), Lebak Saat (2,250 m) and the Aloon-Aloon of Mt. Gede (2,750 m) were used as field bases.

Frogs and toads from Rarahan, a small village just outside the Botanical Garden were included in this report. This area is not forested and it consists of ricefields and vegetable gardens.

About 500 specimens of frogs and toads representing 19 species were collected and were used as the basis of this study. All specimens are deposited at the Bogor Zoological Museum. All measurements were done with a vernier caliper with an accuracy of 0.1 mm.

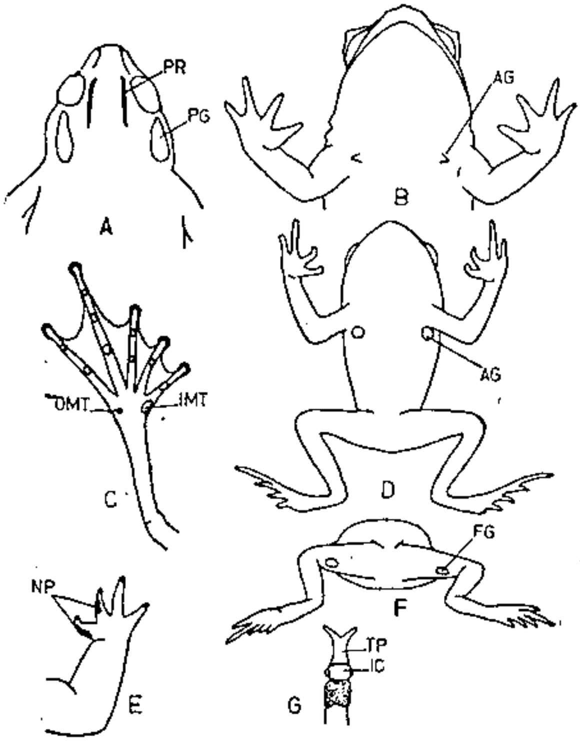

FIG. 1. *A,* Dorsal view of head region of *Bufo biporcatus biporcatus; B,* anteroventral view of *Megophrys monticola monticola; C,* ventral view of the right foot of *Hylarana chalconota chalconota; D,* ventral view of *Leptobrachium hasselti*; *E,* dorsal view of the right forearm of *Bufo melanostictus; F,* Posterior view of *Leptobrachium hasselti; G,* dorsal view of the distal phalanges of the third finger of *Polypedates leucomystax leucomystax; AG,* axillary gland; *FG,* femoral gland; *IC,* intercalary catilage; *IMT,* inner metatarsal tubercule; *NP,* nuptial pad; *OMT,* outer metatarsal tubercle; *PG,* parotoid gland; *PR,* parietal ridge; *ST,* subarticular tubercle; *TP,* terminal phalanx.

(N=3) in snout to vent length. Distinguishable from *asper* by the smaller size and less extensive webbing of toes (toes half webbed in *melanostictus* and fully webbed in *asper*), and from the smaller *biporcatus* by absence of paired parietal ridges on head.

As in any other *Bufo,* skin of the back has large warts, sides with smaller ones, are sometimes tipped with melanic spinules.

Color (in life): Brown or yellowish above, cranial ridges, dorsal warts, and tips of digits tipped with black.

Secondary sex characters.—Females slighty larger than males; mature live males distinguished from females by darker color of throat; presence of dark nuptial spinules on dorsal and medial surfaces of first finger from wrist to tip of finger, and on dorsomedial surface of second and sometimes on third finger (NP, Fig. 1E); presence of a single slitlike opening of vocal sac that may be on right or on left side of mouth floor.

Ecological notes.—*B. melanostictus* is found near human habitations under logs or in gutters of houses during the day and forage under lighted windows and doors of the houses at night. One specimen was caught in a flooded ricefield about 10 m away from the nearest house. This species has never been found in the forest here or any where else in Java. They generally breed in roadside ditches, or small fishponds near houses. Breeding choruses could be heard even during the day; Bufo *melanostictus* has a typical bufonid guttural call.

Remarks.—Compared to the coastal district in Java, *B. melanostictus* is relatively rare in high altitudes. In Tjibodas area it is found primarily in Rarahan but rarely inside the Tjibodas Botanical Garden. This species is the most abundant bufonid in Java, and it occurs in a variety of habitats (except rainforest) from sea level up to 1,500 m above sea level.

BUFO ASPER Gravenhorst.

Bufo asper GRAVENHORST, 1829. Delic. Mus. Zool. Vratislava, fasc. 1, p. 58 – Java; VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 82 (part); INGER, 1966, Amph. Borneo, p. 66.

Material examined.—Tjibodas, 3; Sumatra, 20; Borneo, 4; other parts of Java, 6.

Diagnosis.—Very large *Bufo*, adults about 100 mm long from snout to vent, limbs relatively long. Distinguished from *melanostictus* by the fully webbed toes, and from smaller *biporcatus* by absence of parietal ridges along head; adults appear more slender than stocky *melanosticus* or *biporcatus.*

Color (in life): Dorsum dark brown; ventrally yellowish brown, and usually spotted with irregular black blotches. from throat to thigh.

Secondary sex characters.—Females slightly larger than males. Mature males easily distinguished from females by presence of a pair of slitlike openings of vocal sac on mouth floor, and by presence of black nuptial pads on mediodorsal surface of first and second fingers.

Ecological notes.—*B. asper* occurs along the bank of creeks and small streams and usually burrow themselves under roots of trees or shrubs. The three syllables Kwek-kwak-Kwok call of the males are very characteristic. The males appear not to aggregate in a chorus

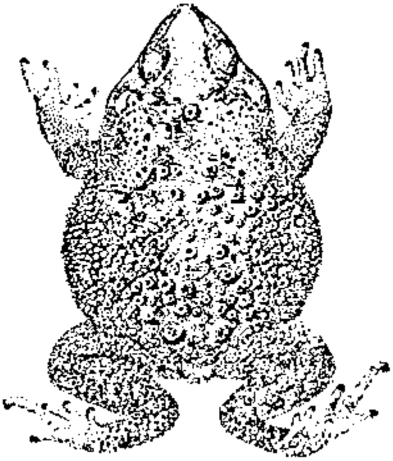

FIG. 2. Dorsal view of *Bufo melanostictus* Schneider.

during breeding. *B. asper* is usually found away from human habitation in cool shaded places; but sometimes it is also found in suburban areas of a big city such as Bandung.

BUFO BIPORCATUS Gravenhorst.

Bufo biporcatus GUNTHER, Cat. Batr. Sal. Brit. Mus., p. 62; VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 90, fig. 9; MERTENS, 1930, Abh. Senck. Naturf. Geselsch **42** (1): 92; INGER, 1966, Amph. Borneo, p. 56.

Bufo parvus VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 88 (part): SCHIJFSMA, 1934, Truebia **14** (1): 47.

Material examined.—Tjibodas, 5; Udjung Kulon, 24; other parts of Java, 18.

Diagnosis.—Small size *Bufo,* adult males ranging from 36 to 45 mm (N=3), while females slgihtly larger 46 to 65 mm (N=2). Body stocky. Distinguished from other Javanese *Bufo* by presence of parietal ridges. Skin, warty as in all *Bufo*, sometimes tipped with melanin. Toes webbed on basal half.

Color (in life): Brownish or yellowish brown above and on sides; dorsum with black irregular markings; hind limbs with dark crossbars.

Secondary sex characters.—Males smaller than females; adult males distinguished from females by presence of vocal sacs, with one slitlike opening on mouth floor. Nuptial pads blackish with minute spinules covering dorsomedial surfaces of first finger; sometimes nuptial spinules also present on medial surfaces of second finger.

Ecological notes.—In Tjibodas *Bufo biporcatus* is restricted to Rarahan, along village clearings or footpaths, but never found in the forest. Inger (1966) found Bornean *biporcatus* in a variety of habitats from village clearings to virgin primary rainforest. In Java at present, this species appears to be rare near human habitation and cultivated areas and this agrees with Church's studies (1960); in Udjung Kulon,[1] however, they are quite abundant in forest clearings. In Java this species occurs from sea level up to 1,500 m above sea level.

CACOPHRYNE CRUENTATA (Tschudi). **Fig. 3.**

Bufo cruentatus Tshudi, 1838, Class Batr., Neuchatel, p. 88; van Kampen, 1923, Amph. Indo-Austr. Arch., p. 78; Schijfsma, 1934, Truebia **14** (1): 44.

Materials examined.—Tjibodas, 149.

Diagnosis.—Small bufonid forest toad, males ranging from 20.0 to 29.8 mm (N=125) and females slightly larger 25.0 to 39.0 mm (N=24) in length from snout to vent. *Cacophryne cruentata* could be distinguished from other Tjibodas toads, by combinations of the following: presence of 2 or 3 pairs of oval parotoid glands behind eyes followed by a series of large tubercles; vent opening at the end of a long tube, directed ventrally near ventroposterior aspect of thighs.

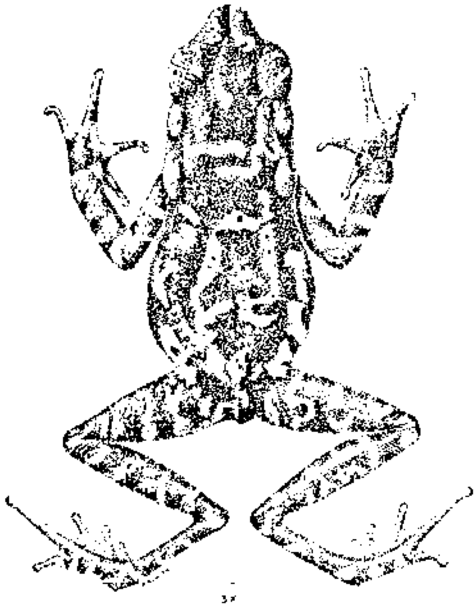

Fig. 3. Dorsal view of *Cacophryne cruentata* (Tschudi).

Color (in life): Dorsum black or brownish black with red spots or reticulations; some specimens with yellowish green reticulations. Red crossbars found on forearm, and sometimes present on hind limbs. A V-shaped black marking present behind eyes. Ventral surface red or yellowish and sometimes with blackish-brown vermiculation on belly and throat. Lower surface of thighs always bright red.

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[1] This is a National Park of lowland rainforest at the most western tip of Java.

Secondary sex characters.—In male, subgular vocal sac opening found on either side of mouth floor. Males also distinguished from females by presence of black nuptial pads on inner surface of first finger or sometimes also on second.

Ecological notes.—This little bufonid occurs in the forest and along small creeks, or streams on boulders and rocks. They are active at night, but some are also seen during the day foraging in shaded banks of creeks. One specimen was collected on the forest floor during a rainy day, far away from the nearest body of water. The call of *Cacophryne cruentata* is a weak, chirping sound. On the 8th of May, 1964, several amplexus pairs were observed in streams under heavy shrubs. Egg masses with large eggs were found in shallow water attached to stones and leaf litter. About a dozen large eggs are clustered in a clear gelatinous egg mass instead of a long gelatinous string as in typical *Bufo* egg mass.

Localities.—*Cacophryne cruentata* is known only from Tjibodas, Mt. Gede, Java. They are found in Tjibeureum waterfall, Rawa denok and the highest elevation recorded was from Lebak Saat, 2,250 m above sea level. No specimen was found near the crater of Mt. Gede as reported by van Kampen (1923).

Family PELOBATIDÆ

In Tjibodas, this family is represented by two genera, *Megophrys* and *Leptobrachium*. These genera could be distinguished from other Tjibodas genera by combinations of the following characters: Arciferous pectoral girdle, toothed maxilla, dilated sacral diapophysis, absence of parotoid gland, and the presence of axillary glands.

MEGOPHRYS MONTICOLA MONTICOLA Kuhl & Van Hasselt. Fig. 4.

Megophrys monticola KUHL & VAN HASSELT, 1882, Isis, 475; MERTENS, 1934; Arch., Hydrobiol.. 12 suppl. (Trop. Binnengew) **4**: 679 (part); INGER, 1954, Amph. Philippines, p. 222; INGER, 1966, Amph. Borneo p. 36 (part).

Megalophrys montana BOULENGER, 1908, Proc. Zool. Soc. London, 1908: 411 (part); VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 8 (part).

Materials examined.—Tjibodas, 56; other parts of Java, 15.

Diagnosis.—Medium-sized megophryd frog; males 33.8 to 48.0 mm (N=16), and females larger 40.0 to 87.0 mm (N=39) from snout to vent length. Easily distinguished from other Tjibodas frogs by presence of triangular dermal appendages on upper eyelids and at commissure of jaws; and by a pair of longitudinal dorsolateral skin folds extending from behind head to about three quarters down length of trunk and connected by a transverse dermal fold on occiput. A pair of white nipplelike axillary glands present between both axilla (Fig. 1B). A few large warts may be present on dorsum. In old specimens, skin of head and anterior part of back co-ossified to the skull.

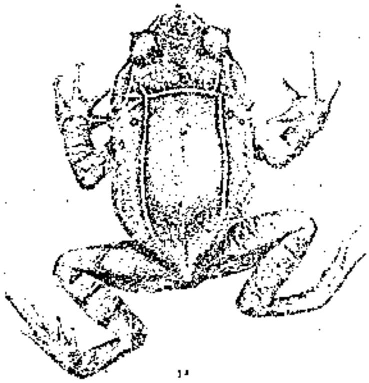

FIG. 4. Dorsal view of *Megophrys monticola* Kuhl & Van Hasselt.

Color (in life): Ground color usually brown or blackish brown, with triangular markings on eyelids and occiput. Dorsal surfaces of hind limbs with dark crossbars. A broad dark band runs from eye to angle of jaws and continues to base of forearm. Ventral surface of body with irregular dark markings; black blotches present on belly and ventral surfaces of thighs; a black streak runs from wrist to midway down along forearm, and another one on ventral surface of elbow; a black blotch also found on knee; ventral surface of tarsus to tips of toes, black. Cloacal region, black, edged with a light streak dorsally. Usually with a light blotch on anteroventral region of eye.

Secondary sex characters.—Adult males distinguished from females by presence of black nuptial pads on mediodorsal surfaces of first and second fingers. All males of *Megophrys monticola monticola* from Java with vocal sacs, contrary to Boulenger's statement (1908) and this agrees with Inger's (1954) observation of one Javanese male specimen. Out of 16 adult males examined, 15 had a pair of slitlike openings and only one had a vocal sac opening on left side.

Ecological notes.—Like other megophryds, *monticola* is a terrestrial frog. Usually it forages on the forest floor at night and also during or after a rainy day. *Mogophrys m. monticola* could only jump for a short sence of triangular dermal appendages on upper eyelids and at commissure of jaws; and by a pair of longitudinal dorsolateral skin folds extending from behind head to about three quarters down length of trunk and connected by a transverse dermal fold on occiput. A pair of white nipplelike axillary glands present between both axilla (Fig. 1B). A few large warts may be present on dorsum. In old specimens, skin of head and anterior part of back co-ossified to the skull.

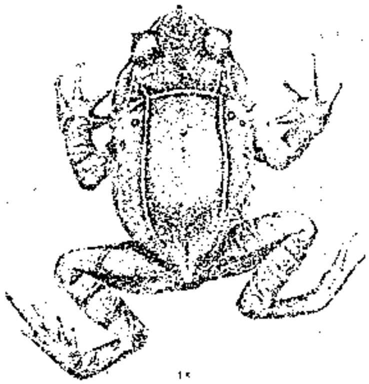

FIG. 4. Dorsal view of *Megophrys monticola* Kuhl & Van Hasselt.

Color (in life): Ground color usually brown or blackish brown, with triangular markings on eyelids and occiput. Dorsal surfaces of hind limbs with dark crossbars. A broad dark band runs from eye to angle of jaws and continues to base of forearm. Ventral surface of body with irregular dark markings; black blotches present on belly and ventral surfaces of thighs; a black streak runs from wrist to midway down along forearm, and another one on ventral surface of elbow; a black blotch also found on knee; ventral surface of tarsus to tips of toes, black. Cloacal region, black, edged with a light streak dorsally. Usually with a light blotch on anteroventral region of eye.

Secondary sex characters.—Adult males distinguished from females by presence of black nuptial pads on mediodorsal surfaces of first and second fingers. All males of *Megophrys monticola monticola* from Java with vocal sacs, contrary to Boulenger's statement (1908) and this agrees with Inger's (1954) observation of one Javanese male specimen. Out of 16 adult males examined, 15 had a pair of slitlike openings and only one had a vocal sac opening on left side.

Ecological notes.—Like other megophryds, *monticola* is a terrestrial frog. Usually it forages on the forest floor at night and also during or after a rainy day. *Mogophrys m. monticola* could only jump for a short distance. They were successfully collected in pit traps by using a one-gallon tin can. An amplexus pair was observed in March 1964. During that time numerous male calls were heard near Tjibeureum waterfall; breeding males do not congregate to form a chorus, but were observed to be far from each other. For this species, it appears that March is the peak of the breeding season for even during daytime, calls were heard frequently. The call is a low Kwok-Kwok-Kwok repeated every 2 to 3 minutes. In Java *M. m. monticola* is a mountain forest frog, but never found outside primary rainforest.

LEPTOBRACHIUM HASSELTI Tschudi. Fig. 5.

Leptobrachium hasselti TSCHUDI, 1838, Class. Amph., p. 81; TAYLOR, 1962, Amph. of Thailand, p. 313; INGER, 1966, Amph. Borneo. p. 29.
Megalophrys hasselti VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 13.
Megophrys hasselti INGER, 1954, Amph. Philippines, p. 213.

Materials examined.—Tjibodas, 12; Udjung Kulon, 25; other parts of Java, 17.

Diagnosis.—Small-sized leptobrachid frog, males ranging from 32.5 to 36.8 mm (N=11) and one female 48.2 mm in snout to vent length. Distinguished from other Tjibodas frogs by presence of flat axillary glands near axilla behind base of arm (AG, Pl. 1D), and rounded femoral glands on posterior surface of each thigh (FG, Pl. 1F). Fingers and toes without webs; orientation of pupil vertical. Differs from *Megophrys monticola* by its smaller size, absence of transverse and dorsolateral skin folds on back.

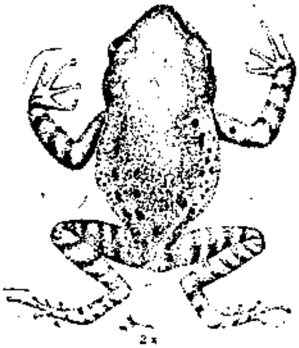

FIG. 5. Dorsal view of *Leptobrachium hasselti* Tschudi.

Color (in life): Dorsum black or dark brown, with indistinct round dorsal blotches; flanks with dark spots; belley and posterior surfaces of thighs granular; throat and belly with dark brown spots. A black streak runs from tip of snout to anterior angle of eye and continues to base of forearm; streaks reddish brown in one specimen; color of both axillary and femoral glands, white cream. Dorsal surfaces of forearms and hind limbs with dark crossbands.

Secondary sex characters.—Females definitely larger than males. Adult males have median subgular vocal sacs with a pair of openings on floor of mouth. Nuptial pads absent.

Ecological note.—Leptobrachium hasselti is restricted in the rainforest. In Tjibodas they are usually found on the forest floor, under shrubs and among leaf litter. *L. hasselti* males were found apart (roughly 10 to 30 m) from each other and never congregating; this observation was based on the calls. Calling frogs were found distant from the nearest body of water. The calls are heard in the evening from 6:00 to 9:00 p.m. Usually frogs crawl, rather than jump, even if disturbed. In Java this species is found in lowland or mountain rainforest.

Family MICROHYLIDÆ

This family is represented by one genus. *Microhyla*, which could be distinguished from other Tjibodas genera by a combination of the following characters: Firmisternal pectoral girdle, sacral diapophysis not dilated, absence of parotoid gland, absence of maxillary teeth, skin of body and limbs smooth; and the relatively small size (less than 30 mm).

MICROPHYLA ACHATINA Tschudi.

Microphyla achatina TSCHUDI, 1838, Class. Batr., p. 71, (type locality, Java); VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 154 (part); PARKER, 1934. Frogs Family Microhylidæ, p. 136.

Materials examined.—Tjibodas, 46; Udjung Kulon, 47.

Diagnosis.—Moderate-sized microphylid frog, males 19.7 to 23.0 mm (N=40), females 24.0 to 26.1 mm (N=6) in snout to vent length. *Microhyla achatina* differs from *palmipes* by its larger size; less extensive webbing of the toes (one third in the former and two thirds in the latter), obtusely pointed snout, and a larger disc of digits.

Color (in life): Dorsum light pinkish brown with a pair of dark brown streaks running parallel from shoulders to groin. A median narrow dark brown stripe with irregular borders run between eyes to coccyx; a white cream streak, edged dorsally with brown, runs from angle of eye to base of forelimbs anterior and posterior surfaces of tarsus and feet dark brown. Ventral region cream-white; throat region spotted with brown. Cloacal region black and outer surfaces of forelimbs with dark brown bands.

Secondary sex characters.—Males with a median subgular vocal sac, with paired openings in 34 individuals and only 12 individuals with a single opening on right side. Gular skin of males wrinkled and bounded posteriorly by a transverse skin fold across breast, just anterior to base of forelimbs.

Ecological notes. M. achatina is found on the banks of quiet pools in Tjibodas Botanical Garden or in water-filled ditches along the roads to Rarahan. This species has not been found in the forest. The males usually call from the base of sedges or in rock crevices along the bank of a quiet pool, or sitting on leaves of water plants. Males aggregate in breeding choruses and calls could be heard throughout the night. Amplexus pairs were observed in May 1964, and a week later surface feeding funnel-lipped microhylid tadpoles were found. In Java this species occurs in a variety of habitats but not in rainforest, from sea level up to 1,500 m above sea level.

MICROPHYLA PALMIPES Boulenger.

Microhyla palmipes BOULENGER, 1897, Ann. Mag. Nat. Hist., (6) 19: 108 (type locality, Pengalengan, Java, 4,000 ft.), VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 157; NIEDEN, 1926. Das Tierreich, Anura II, p. 33; PARKER, 1934, Frogs Fam, Microhylidae, p. 132.

Microhyla annectens VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 156 (part); NIEDEN, 1926, Das Tierreich, Anura II, p. 32 (part).

Materials examined.—Tjibodas, 126.

Diagnosis.—Very small frog, males to 12.4 to 14.4 mm (N=84) and females 13.5 to 18.5 mm (N=42) in snout to vent length. Differs from *M. achatina* by its smaller size and extensive webbing between toes which are two-thirds to three-quarters webbed. Snout rounded, slighty projecting. Terminal segment of digits dilated into a small distinct disc. A very small tubercle usually present on posterior half of upper eyelid. Skin folds present from posterior angle of eye to base of forearm and another dorsal one extending to middle of flank.

Color (in life): Dorsum greenish gray, a brown mark running longitudinally from level of upper eyelids, narrowing just behind head and abruptly widening at level of shoulders and gradually widening until it disappears behind sacral region. Dark brown crossbars present on dorsal surfaces of extremities. Base of hand to axilla, black. An elongated black spot present in front of knee and on heel. Ventral surface of body and posterior surfaces of thighs with black dots. Cloacal region black.

Secondary sex characters.—Males with median subgular vocal sac, paired openings in 32 individuals, while 18 individuals with a single opening on right side. Loose gular skin in males, less conspicuous than in *achatina.* As in other species, females slightly larger than males (see above).

Ecological notes.—In Tjibodas, *Microhyla palmipes* is restricted in the Gajonggong peat bog area which is about half a kilometer south of Tjibeureum waterfall. This small microhylid was easily collected during the day on moist mossy forest floor near Gajonggong peat bogs. (Plate 1, fig. 1). Individuals of this species seem to congregate during the day and if disturbed, jump all over the place to a height of ½, and a linear distance of 3/4 of a m. At first sight they look like jumping crickets. These frogs are active throughout the year, and breeding clusters could be seen in March. Transparent tadpoles, like the one described by Schijfsma (1934), were observed in March, April, and May, 1964. No call has been associated with this species. In Java this species is restricted to rainforest between 1,000 to 2,000 m above sea level.

Family RANIDÆ

In Tjibodas this family is represented by three genera, namely: *Rana, Hylarana,* and *Amolops.* This family is characterized by the following characters: Firmisternous pectoral girdle; sacral diapophysis not dilated; maxillary teeth present; intercalary cartilage absent; parotoid gland absent; and skin of body and limbs not warty.

Genus RANA Linnaeus

The five species of Tjibodas *Rana* are characterized by the following combination of characters: absence of parotoid, axillary, or femoral glands; tips of digits not or only slightly dilated; absence of circummarginal groove between upper and lower surfaces of tips of digits.

RANA CANCRIVORA CANCRIVORA Gravenhorst.

Rana cancrivora cancrivora GRAVENHORST, 1820, Delic. Mus. Zool. Vratisl 1: 41 (type locality, Java); VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 170; TAYLOR, 1962, Univ. Kansas Sci. Bull. **43** (8): **377**.

Rana cancrivora cancrivora INGER, 1954, Fieldiana-Zool. **33**: 260, figs. 49-50; 1956, *ibid.* **34**: 401; 1966, *ibid.* **52**: 175:

Materials examined.—Tjibodas, 12; other parts of Java, 21.

Diagnosis.—Medium to large-sized frog; males 58.4 to 71.6 mm (N=8), females 46.2 to 92.3 mm (N=4) in snout to vent length. *Rana c. cancrivora* differs from *limnocharis* by absence of the outer metatarsal tubercle and more extensive webbing (reaching most distal supernumerary tubercle of fourth toe); and from *kuhli* by distinct tympanum; and from *microdisca* by the simple unexpanded tip of digits. Distinct fringe of skin present on outer side of fifth toe and metatarsal, and presence of irregular longitudinal skin folds on back.

Color (in life): Brownish green with irregular dorsal dark brown markings; lips with vertical dark brown bars; dorsal surfaces of limbs with dark brown or blackish crossbars. Ventral surface cream, and sometimes with dark mottling.

Secondary sex characters.—Females larger than males. Males with median subgular vocal sacs with a pair of openings; breeding males with wrinkled loose skin on sides of throat, and a nuptial pad present on first finger. Males distinguished by black patches at corners of throat; but these two black patches never confluent across throat thus differing from *limnocharis* males which have characteristic M-shaped black marking on throat.

Ecological notes.—As in other parts of Java, the Tjibodas *cancrivora* occurs near human habitation; in Rarahan they were observed along roadside ditches, fishponds and ricefields. This species has not been found in the forest nor in the Tjibodas Botanical Garden. This species is more abundant in the lowland than in higher altitudes.

Remarks.—Rana c. cancrivora as well as *Rana m. macrodon* are regularly collected in Java and sold in the market for human consumption. In Java *cancrivora* is found in a variety of habitat (except rainforest), from sea level up to 1,500 m above sea level. This species and *limnocharis* are the two most common *Rana* in Java.

RANA LIMNOCHARIS LIMNOCHARIS Boie.

Rana limnocharis Boie in WIEGMANN, 1835, Nova Acta Acad. Leop. Carol. **17**: **255** (type locality, Java); VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 167, fig. 23; TAYLOR, 1962, Univ. Kansas Sci. Bull, **43** (8): 380, fig. 35; INGER, 1966, Fieldiana-Zool. **52**: 205.

Materials examined.—Tjibodas, 12; other parts of Java, 128.

Diagnosis.—Moderate-sized ranid; adult males 37.7 to 46.7 mm (N=6) and females 32.9 to 51.9 mm (N=6) in snout to vent length. Snout pointed; skin flaps along outer sides of fifth toe and metatarsal absent, and webbing on fourth toe reaches only second subarticular tubercle. Dorsum with irregular longitudinal ridges. *Rana l. limnocharis* differs from *kuhli* by the visible tympanum, from *microdisca* by the simple unexpanded tip of digits and the presence of the outer metatarsal tubercle.

See under species diagnosis of *cancrivora* for differences with *limnocharis.*

Color (in life): Dorsum olive to greenish-brown with irregular dark brown bar running across interorbital area forming an angle at midline. Usually three dark brown vertical bars on upper and lower lips present; a median longitudinal light brown or somewhat orange stripe usually present from tip of snout to end of coccyx. Dorsal surfaces of extremities with dark cross bars. Ventral surfaces yellowish to cream; in males, an M-shaped dark patch on posterior part of throat present.

Secondary sex characters.—Adult males characterized by a distinct M-shaped black marking on throat. Adult males with a median subgular vocal sac with a pair of openings, and a nuptial pad on first finger.

Ecological notes.—Both *Rana cancrivora* and *R. limnocharis* have never been found in forests. They are usually found in roadside ditches, fishponds, ricefields or in any temporary pools. In Tjibodas, *limnocharis* is found outside the National Park and the Botanical Garden. In Java *limnocharis* is sympatric with *cancrivora* and shares the same habitats.

RANA KUHLI Dumeril and Bibron. Fig. 6.

Rana kuhli DUMERIL and BIBRON, 1841, Erp. Gen. **8**: 384 (type locality, Java); VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 178; INGER, 1956, Fieldiana-Zool. **34**: 403; TAYLOR, 1962, Univ. Kansas Sci. Bull., **43** (8): 408; INGER, Fieldiana-Zool. **52**: 196.

Materials examined.—Tjibodas, 101.

Diagnosis.—A moderate-sized stocky *Rana*, limbs strong and short (Fig. 6). Males 40.4 to 84.2 mm (N=60) and females 40.3 to 66.6 mm (N=41) in snout to vent length; R. *kuhli* could be distinguished from other Tjibodas *Rana* by the following combinations of characters: tympanum covered by skin hence not visible; toes entirely webbed to tips of digits; a pair of tusks present near mandibular symphysis,

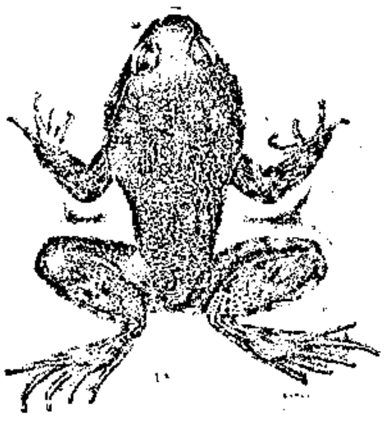

FIG. 6. Dorsal view of *Rana kuhli* Dumeril et Bibrōn.

and tips of toes distinctly expanded but without the circummarginal grooves. Tarsal folds present; heels when folded at right angle to body, not overlapping. Outer metatarsal tubercle absent and inner metatarsal tubercle oval. Dorsum with short longitudinal folds or rounded warts, sometimes, smooth. Dorsal surfaces of tibia spinulated. Ventral surface smooth. Pupil rhomboidal and bordered by yellow; eyes with dark olive reticulations.

Color (in life): Dorsum dark olive and sometimes with black spots; a straight dark olive interorbital bar bordered anteriorly by a narrow yellowish green bar. A black streak from behind eye to base of forearm present. Usually, a short longitudinal reddish brown skin fold present behind eye. Lips usually with vertical dark brown bars; throat white but mottled with brownish black.

Secondary sex characters.—Mature males without vocal sacs; nuptial pads present on first finger; males differ with relatively larger head and larger tusks on mandible than females.

Ecological notes.—In Tjibodas this species is restricted in the forest. Out of 101 specimens collected only two were collected in a drainage ditch in Tjibodas Botanical Garden. *R. kuhli* is usually found in stagnant or slow-moving water in shaded places. It is aquatic and secretive. Usually it escapes by diving into the water and hiding under rocks. This frog was never collected away from water. No call has been associated with this species. *R. kuhli* is a mountain forest frog, and in Java, it occurs above 700 m from sea level.

RANA MICRODISCA MICRODISCA Boettger.

Rana microdisca BOETTGER, 1892, Ber. Offenb. Ver. f. Naturk (1887-1891), p. 137 (type locality, Java); VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 180 (part); INGER, 1954, Fieldiana-Zool. **33**: 290 (part); 1966, *ibid.*, **52**: 212 (part)

Rana microdisca microdisca MERTENS, 1934, Arch. Hydrobiol., Suppl. Band. **12** (Trop. Binnengew) **4**: 683; MERTENS, 1957, Treubia **27**: 86.

Rana hascheana VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 181 (part).

Materials examined.—Tjibodas, 30; Udjung Kulon, 30; Pengandaran, 1.

Diagnosis.—A moderate-sized *Rana;* males 25.8 to 41.0 mm (N=15) and females 34.4 to 48.7 mm (N=15) in snout to vent length. This frog could be distinguished from other *Rana* in Tjibodas by the following combinations of characters: tympanum distinct; tips of digits dilated, but without the circummarginal groove; toes webbed on basal usually two distal phalanges of fourth toe free of webbing; tarsal folds absent; heels strongly overlapping when folded at right angles to body; dorsum with some rounded or short longitudinal skin folds;

ventral surfaces smooth; cloacal region and ventral surfaces of thighs slightly granular. Pupil orientation horizontal and rhomboidal.

Color (in life): Dorsum greenish or reddish brown; a dark brown interorbital bar present between eyes, and bordered anteriorly by a yellowish-red band. A W-shaped dark brown marking present on back between forearms. A black streak runs from eye over tympanum; upper and lower lips with white and dark brown vertical bars; dorsal surfaces of limbs with dark brown cross-bars. Ventral surface of body cream or yellow; ventral side of thighs pink or orange-yellow; dorsal side of toe discs orange. Eye yellow with brown reticulations; pupil reddish brown; a vertical brown band present on eye.

Secondary sex characters.—Tjibodas and other Javanese adult males lack vocal sacs and nuptial pads. The only difference between the sexes is that females are slightly larger than males.

Ecological notes.—In Tjibodas, *R. microdisca* is restricted to the forest, usually found in a temporary pool or stagnant water. This species is never found in swift moving creeks or streams. No call has been associated with this species. In Java, *microdisca* is found from sea level in Pengandaran and Udjung Kulon but up to 1,500 m above sea level in Tjibodas.

HYLARANA CHALCONOTA CHALCONOTA Schlegel.

Hyla chalconotus (part) SCHLEGEL, Abbild. neuer order un vollst bek. Amph., Dusseldorf, 1837-44, p. 23, (type locality, Java.).

Polypedates Junghuhnii BLEEKER, 1856; Nat. tijdsch. Med-Indie **11**: 469.

Rana chalconota VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 217; SCHIJFSMA, 1932, Treubia **14** (1): 64; INGER, 1966, Fieldiana-Zool. **52**: 177.

Materials examined.—Tjibodas, 215; Udjung Kulon, 2; Surabaya, 3; and Bandung, 24.

Diagnosis.—Moderate to large-sized *Hylarana;* males 37.4 to 47.8 mm (N=121) and females 44.1 to 71.0 mm (N=94) in snout to vent length. This species is characterized by the following combination of characters: Tips of fingers and toes distinctly dilated, discs with circummarginal grooves; first finger shorter than second; web reaching discs of first, second, third and fifth toes, whereas distal phalanx of fourth toe not webbed (Fig. 1C); two metatarsal tubercles present, an oval inner and a round outer one; heels overlapping when folded at right angles to body. Snout pointed triangular in shape; dorsolateral skin fold present behind eye; another skin fold present from below eye to base of arm, followed by one or two elongated oval grandular patches. Dorsum smooth or finely granular, sometimes with some warts. Posterior part of belly and ventral sides of thighs granular. Pupil oval and oriented horizontally.

Color (in life): Dorsum greenish yellow or olive, sometimes with brown spots; dorsolateral gland and gland behind rictus yellow. A dark brown streak present from snout to anterior rim of eye; upper lip whitish cream; dorsal side of thighs with or without brownish gray crossbars; ventral side of thighs and posterior part of abdomen reddish pink; sometimes webbing of toes also reddish. Eyes yellowish orange. Nuptial pads in males yellowish gray.

Secondary sex characters.—Adult females conspicuously larger than males (see above). Smallest male in amplexus 36.6 mm; female 55.3 mm in snout to vent length. Adult males distinguished from females by the following: subgular vocal sacs with a pair of round openings on floor of mouth (in preserved specimens openings constricted); nuptial pads present on medio-dorsal surfaces of first fingers; sometimes a very small humeral gland present (less than 3 mm long).

Ecological notes.—In contrast with *Hylarana c. raniceps* of Borneo (Inger, 1966), which is a forest species; the Javanese *H. chalconata* does not occur in the forest except in Udjung Kulon. In Tjibodas *c. chalconota* has not been found in the forest. It is common in the Tjibodas Botanical Garden, along roadside ditches. in pools and over hanging vegetation 1.5 m above water. In Java, this species is associated with human habitations and frequently enters houses; it is one of the commonest frogs and breeding choruses occur even during the day. In Java, it occurs from the coast up to 1,500 m above sea level.

HYLARANA NICOBARIENSIS Stoliczka.

Hylorana nicobariensis Stoliczka, 1870, Jour. Asiatic Soc. Bengal **39**: 150 (type locality, Nicobar).

Rana nicobariensis van Kampen, 1923, Amph. Indo-Austr. Arch., p. 224; Inger, 1966, Fieldiana-Zool. **52**: 223.

Materials examined.—Tjibodas, 11; Telga Patengan, 2; Tjiapus, 1; and Pulau Peutjang, 1.

Diagnosis.—A very slender frog; males 33.2 to 37.1 mm (N=10) and a female 39.3 mm in snout to vent length; head elongated; snout pointed (longer than eye diameter) and projecting. Fingers and toes distinctly dilated with circummarginal groove between dorsal and ventral surfaces of discs; discs of fingers less than half width of tympanum; web on firts, second, third and fifth toes reaching midway between digital discs and most distal subarticular tubercle. Fourth toe with three distal phalanges not webbed. A pair of metatarsal tubercle present, a round inner and an oval one. Heels overlapping each other when folded at right angles to body. Dorsum and flank granular; dorsolateral glandular fold narrow; glands on rictus present; ventral surface smooth; ventral sides of thighs granular. *H. nicobariensis* similar to *Amolops jerboa* and *H. chalconota* on having a slender body and presence of dorsolateral glandular fold; *nicobariensis,* easily distinguished from *chalconota* and *jerboa* by the narrow web between toes, the less extensive webbing of fourth toe, and smaller discs of fingers (less than half diameter of tympanum).

Color (in life): Dorsum light brown with dark brown spots; lateral sides of head and body dark brown and spotted near groin. Dorsolateral glandular fold, upper lip and glands behind rictus silver white. Dorsal sides of thighs with dark brown crossbands, dorsum of forelimbs and tibia with dark brown spots. Ventral side white.

Secondary sex characters.—Female larger than males; adult males with paired subgular vocal sacs and a pair of round openings on floor of mouth and a nuptial pad on dorsomedial surface of first finger. In addition males distinguished from females by presence of a large oval humeral gland (about four-fifths length of upper arm).

Ecological notes.—In Tjibodas area this species is found outside the rainforest or dense secondary growth, and usually in standing or slow-moving water. In Java, *nicobariensis* is found primarily in the ricefields. The harsh call (kek, kek, kek, repeated 4 to 6 times) could be heard not only at night but also throughout the day. In Java it occurs from sea level to 1,500 m above sea level.

AMOLOPS JERBOA Gunther. Fig. 7.

Hylorana jerboa GUNTHER, 1872. Proc. Zool. Soc. London, p. 599 (type locality, Matang, Serawak).

Rana jerboa VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 208; Schijfsma, 1932, Treubia **14** (1): 63.

Rana whiteheadi VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 210 (part).

Amolops jerboa INGER, 1966, Fieldiana-Zool. **52**: 263.

Materials examined.—Tjibodas, 43.

Diognosis.—Body slender and hindlimbs extremely long (Fig. 7); adult males 25.5 to 46.2 mm (N=27) and females 46.5 to 74.7 mm (N=16) in snout to vent length; head triangular, and obtusely pointed; tympanum conspicuous; tips of fingers and toes dilated with circummarginal grooves; discs of fingers about half diameter of tympanum; feet broadly webbed and reaching discs of all toes; a pair of metatarsal tuber-

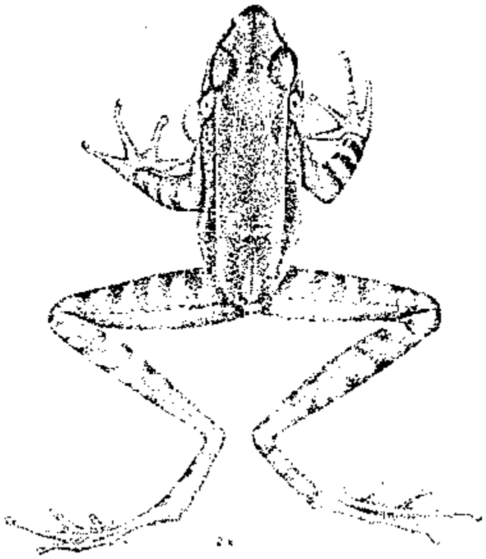

FIG. 7. Dorsal view of *Amolops jerboa* Gunther. Vocal pouches are inflated.

cles present, an oval inner and small round outer one; tarsal fold absent; tibia 0.7 of snout to vent length; heels overlapping when folded at right angle to body; dorsolateral glandular skin fold and another skin fold from above tympanum to base of forelimbs present. Ventral surfaces smooth. Pupil oval and oriented horizontally.

Color (in life): Dorsum light brown; dorsolateral skin fold yellow or olive, bordered ventrally with black; a dark brown streak runs along sides of head from tip of snout to just behind tympanum. Yellowish gland ventral to tympanum present. Dorsal surfaces of limbs with dark brown cross-bars; in males anterior surface of base of forearm dark brown; outer surface of tarsus and fifth toe black. Webs between toes dark brown; ventral surfaces whitish cream and abdomen sometimes yellow; throat and sometimes pectoral region dusted with dark brown spots. Pupil black; eye dark brown with a yellowish green dorsal segment.

Secondary sex characters.—Males considerably smaller than females (see above). Adult males with a pair of subgular vocal sacs on lateral corners of throat, and held by loose skin vocal pouches (Fig. 7); furthermore adult males have gray brown nuptial pads on mediodorsal surfaces of first fingers and sometimes also on second.

Ecological notes.—*Amolops jerboa* is restricted to forests along swift or fast-moving mountain streams. They usually sit on rocks, boulders or vegetation along streams. Out of the 43 individuals collected, only one was taken from Tjibodas Garden a few hundred meters away from a nearby swift-moving stream. This species occurs up to 2,000 m above sea level in Mt. Gede. In Java, the habitat of this species is in swift mountain streams between 1,000 to 2,000 m above sea level. A loud high-pitched call (chiuut) of males is the characteristic call of this frog along the noisy swift running water.

Family RHACOPHORIDÆ

In Tjibodas this family is represented by three genera, namely: *Rhacophorus, polypedates,* and *Philautus.* Rhacophoridæ is characterized by the following combinations of characters: pectoral girdle firmisternous; sacral diapophysis not dilated; vertebræ either procoelous throughout or seventh vertebra amphicoelous; vomerine teeth present or absent; maxillary teeth always present; terminal phalanx of digits bifurcate or Y-shaped. The distinguishing character of this family is the presence of an intercalary cartilage between the two distal phalanges of the digits (1C, fig. 1G). Distal segment of the digits is always distinctly dilated and has a ventromarginal groove. Generally they are arboreal. Members of *Polypedates, Rhocophorus, Chirixalus,* and *Buergeria* produce frothy nests for egg-laying. Four species representing three genera are found in Tjibodas.

RHACOPHORUS REINWARDTI Kuhl and van Hasselt.

Rhacophorus reinwardti KUHL en van HASSELT, 1822, Alg. Konst-en Litterbode. p. 104.

Hypsiboas reinwardtii BOIE, WAGLER, 1830, Naturl. Syst. Amph., Munchen, p. 200.

Polypedates reinwardtii. SIEDLECKI, 1909, Biol. Centr. **29**: 704; WOLF, 1936, Bull. Raffl. Mus. **12**: 211.

Rhacophorus reinwardti. VAN KAMPEN, 1823, Amph. Indo-Austr. Arch., p. 264: SCHIJFSMA, 1932, Truebia **14** (1): 68; LIEM, 1970, Fieldiana-Zool. **57**: 100.

Materials examined.—Tjibodas, 21; Bogor, 3; Pasirdatar, 1; Tjiseureuh 1; Djampang Udik, 1.

Diagnosis.—Moderate to large-sized tree frog; males 46.0 to 52.1 mm (N=14) and females 61.2 to 74.3 mm (N=7) in snout to vent length; snout rounded; interorbital space broader than upper eyelid; tympanum distinct; fingers and toes with large discs; discs of third or fourth finger larger than diameter of typanum; digital pad completely surrounded by a deep groove referred to as ventromarginal groove (Liem, 1970). Fingers and toes broadly webbed reaching discs, except first finger; only a small inner metatarsal tubercle present; dorsum smooth; abdomen, flank and lower surfaces of thighs granular; a skin fold present above tympanum; a continuous dermal flap runs along outer edge of forearm and fourth finger; a similar flap runs along outer edge of tarsus and along fifth toe; a rounded or rectangular dermal flap present on heel; a horizontal dermal flap present on coccyx.

Rhacophorus renwardti could be distinguished from other Tjibodas frogs by the following combination of characters: intercalary cartilage between two distal phalanges of digits present; web on fingers and toes reaching digital pads (except on first finger); dermal flap on heel rounded or rectangular and thus differing from triangular dermal heel flap of *Rhacophorus javanus.*

Color (in life): Dorsal side of body and extremities leaf green (purplish is preserved specimens); flank orange; ventral side of body yellowish orange; pectoral region whitish; throat bright yellow speckled with green or gray color along the outer margins. Supra-anal flap and dermal heel flap edged with whitish or yellowish edge, flaps along outer sides of forearm, fourth finger, tarsus and fifth toe yellowish white; a dark blue spot present on anterior side of axilla; dorsal side of fourth finger, part of third finger and fifth toe green, orange on the 1st and 2nd toes; digital discs orange; web between three outer fingers, and between four outer toes dark blue to blackish, with longitudinal light blue or grayish blue stripes; eye gray with light brown reticulations.

Secondary sex characters.—Males distinctly smaller than females; adult males with a subgular vocal sac and a pair of round openings on the mouth floor; nuptial pads present on medio-dorsal surface of first finger of males.

Ecological notes.—In Tjibodas *Rhacophorus reinwardti* has not been in the forest; this species is a true tree-dweller; it is usually found 2 to 3 m high in trees or shrubs along quiet pools. In Tjibodas they are not found below 1½ m high from the ground, in contrast with *Rhacophorus jabanus* which occupies the lower stratum (below 1½ m high); In Java, *reinwardti* is found near human habitation, usually occupying moist-shaded areas. The call is a low loud croaking sound usually accompanied by a knocking sound: krok-tok-tok-tok-tok. This species has an extensive vertical distribution, from sea level (in Jakarta) up to 1,500 m above sea level in Tjibodas.

RHACOPHORUS JAVANUS Boettger.

Rhacophorus moschata (*nomen nudum*), KUHL en VAN HASSELT, 1922, Alg. Konst. en leterbode, p. 104 (type locality, Java).

Rhacophorus javanus BOETTGER, 1893, Zool. Amph. Indo-Austr. Arch., p. 254; SCHIJFSMA, 1932, Treubia **14** (1): p. 66.

Polypedates javanus BARBOUR, 1912, Mem. Mus. Comp. Zool. Harvard Coll. **44** (1): 68.

Rhacophorus schlegelii margaritifer WOLF, 1936, Bull. Raffl. Mus. **12**: 187.

Materials examined.—Tjibodas, 105.

Diagnosis.—Moderate-sized tree frog; males 33.2 to 47.6 mm (N=92) and females 57.0 to 68.7 mm (N=14) in snout to vent length; interorbital space slightly broader than upper eyelid; web on three outer fingers reaching midway between second subarticular tubercle and digital discs; first finger webbed at base; discs of third finger 1½ times diameter of tympanum; toes webbed to discs, except distal phalanx of fourth toe digital pads completely surrounded by ventromarginal grooves. A small inner metatarsal tubercle present; dorsum smooth; abdomen and ventral side of thighs granular; a skin fold above tympanum; a dermal ridge present along outer side of forearm, fourth finger and fifth toe (not as extensively as *R. reinwardti*); a low crenulated fringe or a row of tubercles present along outer edge of tarsus; heel flaps triangular; a horizontal supra-anal fringe present or a row of tubercles arranged horizontally; ventral region of the vent granular. Differs from *reinwardti* by the presence of a triangular heel flap and from *Polypedates leucomystax* by the more extensive webbing of the fingers.

Color (in life): Dorsum brown, grayish brown, or yellowish green; irregular dark brown or black markings on back; yellow or white spots present on flank and back; a pair of rounded black spots usually present on sacral region; a straight dark brown orbital transverse bar always present, sometimes, with another one just behind it. Hindlimbs and forearm with brown crossbars; dermal fringes of limbs and outer digits yellowish or white; dorsal side of supra-anal flap yellowish white or with a horizontal row of rounded yellow spots; triangular heel flap yellow or whitish-yellow (in preserved specimen gray); ventral side white or yellowish; ventroproximal part of femur reddish (faded in preserved specimens); dorsal side of first and second finger yellowish; posterior and of thighs reddish with brownish reticulations; web of toes reddish; white spots sometimes present below vent. Pupil black, eye bright yellow with brownish reticulation.

Secondary sex characters.—Males considerably smaller than females; adult males with a subgular vocal sacs and with a pair of round openings on mouth floor; in males nuptial pad present on medio-dorsal side of first finger.

Ecological notes.—*Rhacophorus javanus* is found in Tjibodas Botanical Garden as well as in the forest, usually found pools or slow moving water, on water plants or shrubs. They have a melodious call: kreek, kreek. kreek. In Tjibodas Botanical Garden *R. javanus* shares breeding pools with *R. reinwardti,* the former occupies the lower stratum (less than 1 m from the ground) whereas the latter occupies the upper stratum (1 to 3 m above the ground). In the forest, however, where *reinwardti* is absent *javanus* could be found up to 2 m above the ground. *R. javanus* is found in forested areas and rarely in open cleared agricultural land such as in Rarahan. This species is a montane frog and reach as high as 1,700 m above sea level in Java. Although van Kampen (1923) and Schijfsma (1932) reported this species from Buitenzorg (= Bogor) it could not be found there at present (1963-1964). Apparently *R. javanus* became extinct in Bogor due to human interference of its forest habitats. This species builds a foam nest on vegetation over water (Plate 2, fig. 1) for egg-laying.

Range.—Known only from Java: Tjisurupan, Mt. Malabar (van Kampen, 1923; Schijfsma, 1932). Tjibodas: Rarahan, Tjibodas Garden, Tjibatulawang, Gojonggong, and Tjibeureum.

POLYPEDATES LEUCOMYSTAX LEUCOMYSTAX Boie.

Hyla leucomystax Boie in GRAVENHORST, 1892, Delic. Mus. Vrat., fasc. **1**: 26 (type locality, Java).

Rhacophorus sexvirgata VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 246; SCHIJFSMA, 1932, Treubia **14** (1): 65; INGER, 1954, Fieldiana-Zool. **33**: 376 (part).

Rhacophorus leucomystax var. *sexvirgata* VAN KAMPEN, 1923, Amph. Indo-Austr. Arch., p. 249.

Rhacophorus leucomystax leucomystax WOLF, 1936, Bull. Raffl. Mus. **12**: 178 (part); INGER, 1966, Fieldiana-Zool. **52**: 307.

Materials examined.—Tjibodas, 6; Udjung Kulon, 4; Djakarta, 3; Bogor, 8; Surabaya, 8; and other parts of Java, 41.

Diagnosis.—Large-sized tree frog; a male is 48.2 mm and females 55.0 to 65.8 mm (N=5) in snout to vent length; snout pointed and projecting; interorbital space 1½ to 2 times as broad as upper eyelid; skin of frontoparietal, nasal and squamosal region coossified with skull in individuals of more than 38 mm in snout to vent length. Discs of fingers just a little smaller than diameter of tympanum; discs of digits with ventromarginal grooves; web between fingers never reaching first subarticular tubercles; web on toes reaching discs of first, second, third and fifth toes; two distal phalanges of fourth toe webless; inner metatarsal tubercle present, sometimes an outer one present; abdomen and ventral and posterior side of thighs granular; a dermal fold running from behind eye over tympanum to base of arm; supra-anal and dermal heel flaps or fringes along foot absent; a row of very weak tubercles on outer edge of forearm sometimes present, none on tarsus. *P. leucomystax* could be distinguished from other Tjibodas frogs by the following features, presence of intercalary cartilage, presence of vomerine teeth, skin of head coossified with skull in adults, absence of heel and supra-anal dermal appendages, absence of fringes or flap along outer edges of forearm tarsus, fourth finger and fifth toes.

Secondary sex characters.—Males considerably smaller than females (see above); males with a subgular vocal sac, and two round openings on the mouth floor; nuptial pads present on mediodorsal side of first and second fingers.

Color (in life): Very variable; dorsum gray, yellowish brown or sometimes yellowish green, may be spotted with brown or black with an hour glass marking or with four dark brown longitudinal bands (a pair running from tip of snout to coccyx and the other pair from upper eyelids to groin); a lateral dark brown streak sometimes present from behind eye to midway along flank; dorsal side of forearm, thighs and tarsus with dark brown crossbars; ventral side whitish or cream, with dark spots on throat; both upper and lower lips whitish; nuptial pad whitish.

Ecological notes.—Polypedates leucomystax is the most common tree frog in Java as well as in other parts of the Orient. In Tjibodas, however, this species is rare and only two individuals were collected in the Tjibodas Botanical Garden, four in Rarahan and none in the forest. In the lowland in which this species is abundant it usually occurs near human habitations, in cultivated land around fish ponds or permasent pools. The whitefrothy egg mass is laid on leaves of vegetation over water or sometimes it is laid on the bank of quiet pools. After the eggs hatch the tadpoles fall or are washed away by rain into the pool. The frothy nests are formed when the hindlimbs beat the gelatinous secretion while the pair is in amplexus (Liu, 1950). In Java *leucomystax* has a very extensive vertical distribution from the coast up to 1,500 m above sea level.

Range.—Udjung Kulon, Djakarta, Depok, Bogor, Pasirdatar, Megamendung, Pelabuan, Bandung, Tjimahi, Monosobo, Semarang, and Surabaya. Outside Java: Bawean Isl., Kangean Isl., Panaitan Isl., Sumatra and Borneo. Tjibodas: Tjibodas Botanical Garden and Rarahan.

PHILAUTUS AURIFASCIATUS Kuhl en van Hasselt.

Hyla aurifasciata Kuhl en VAN HASSELT, 1822, Alg. Konst-en letterbode, p. 104 (type locality, Java).

Hyla aurifaciatus SCHLEGEL, 1837, Abbild. Amp., p. 27, pl. 9, fig. 4 (type locality, Java).

Philautus aurifasciatus VAN KAMPEN, 1923, Amph. Indo-Austr., p. 276; INGER, 1966, Fieldiana-Zool. **52**: 341.

Materials examined.—Tjibodas, 18; Telaga Warna, 1; Situ Lembang. 7 (personal collection).

Diagnosis.—Small-sized tree frog; males 15.4 to 21.9 mm (N=10) and females 15.2 to 28.4 mm (N=8) in snout to vent length; snout obtusely pointed; canthus rostralis sharp and curved; nostril midway between anterior rim of eye and tips of snout, or slightly nearer to tip of snout; interorbital space broader than upper eyelid; tympanum indistinct (covered by skin) and about half diameter of eye; tips of fingers and toes with round discs surrounded by ventromarginal grooves; fingers without web except for rudimentary web between first and second fingers. Web between toes not extensive, reaching proximal subarticular tubercles of first and second toes, distal subarticular tubercle of third and fifth and middle subarticular tubercles of fourth toe; only inner metatarsal tubercle present; dorsum with tubercles or short ridges; a skin fold from eye over tympanum and continues to axilla; usually a short dorsolateral ridge present. Pupil oval or rhomboidal (when constricted) and oriented horizontally. This species could be distinguished from other Tjibodas frogs by the following combination of characters: a small-sized frog with an intercalary cartilage between the two distal phalanges of digits, expanded disc with ventromarginal groove and absence of vomerine teeth.

Color (in life): Dorsal coloration variable grayish green, olive or brownish green; with bright yellowish-green middorsal markings or a dark brown H-shaped marking; side of head dark brown or blackish; dorsal side of limbs with dark brown crossbars; ventral side cream or whitish with brown spots on throat and abdomen; pupil black, eyes brown with yellow upper segment.

Secondary sex characters.—Males slightly smaller than females. Males with a subgular vocal sac and two round openings on mouth floor; in males, nuptial pad present on mediodorsal side of first finger.

Ecological notes.—*Philautus aurifasciatus* is only found in the forests and away from streams or pools. Based on the call, the males appear to be scattered in the forest around 20 to 30 m apart, among shrubs that are 1½ to 2 m above the ground. The clear metallic call (Kunk, Kunk) is characteristic of this species. On one occasion 30 to 40 active individuals of this species were observed to congregate in a low shrub during the day. In Java this species is reported as a mountain frog and in Tjibodas it reaches up to 1,800 m above sea level.

Remarks.—All specimens collected from Tjibodas fall within *Philautus aurifaciatus* variations described by van Kampen (1923) and Inger (1966). Since *Philautus aurifasciatus* is such a variable species it will not be surprising if the following species are synonyms of *aurifasciatus*: *Nyctixalus margariffer* Boulenger (Barbour 1912), *Nyctixalus robinsoni* Annandale, and *Philautus pallidipes* Barbour (van Kampen 1923; Barbour 1908, 1912), all reported from Tjibodas but of uncertain status.

HABITAT

The Tjibodas frogs and toads are roughly classified according to habitats in Table I, and their breeding (*b*) and feeding (*f*) activities in the various habitats are designated. Breeding habitats are determined by the presence of either the egg masses, tadpoles, amplexus pairs, or breeding choruses.

Tjibodas frog and toads may be divided into the forest and lowland dwellers. Out of the 19 species of frogs and toads found in Tjibodas area, 10 are forest dwellers and 9 are lowland dwellers. *Hylarana chalconota* and *Bufo biporcatus* were observed outside the forest in Tjibodas and most parts of Java except in Udjung Kulon National Park[2] where these two species were found in the rainforest. In Borneo, the same species were reported by Inger (1966) to dwell in primary rainforest as well as in secondary growth. Apparently these two species were formerly forest dwellers in Java but after severe forest destruction they became adapted to secondary growth forests and lowland habitats.

ALTITUDINAL DISTRIBUTION

The study area located in the south-eastern slope of Mt. Gede and with an elevation of 1,250 to 3,000 m above sea level has provided valuable information on the altitudinal distribution of the Tjibodas frogs and toads. Data on the altitudinal distribution were based on specimens collected or from the call by cruising along streams, peat swamps and through the forest. Various biotic or physical factors appear to affect the distribution pattern of a species but sometimes their effects are not obvious. In this study, the vegetation appears to play a major role in the distribution of frogs and toads in Tjibodas.

[2] A lowland rainforest on the most western tip of Java.

TABLE 1.—*Habitats of Tjibodas frogs and toads.*

		Forest species	**Lowland species**
Terrestrial		*Megophrys monticola (f)* *Leptobrachium hasselti (f)* *Microhyla palmipes (f)*	*Bufo melanostictus (f), B. biporcatus (f)*
Aquatic	**Swift-moving stream**	*Amolops jerboa (f,b)* *Cacophryne cruentata (f)*	*Bufo asper (f,b)*
	Slow-moving stream	*Rana kuhli (f,b), R. microdisca (f,b)* *Cacophryne cruentata (f)* *Megophrys monticola (b)* *Leptobrachium hasselti (b)*	*Rana cancrivora (f,b), R. limnocharis (f,b)* *Hylarana chalconota (f), H. nicobariensis (f)* *Bufo melanostictus (f), B. biporcatus (f)*
	Standing water	*Rana kuhli (f,b), R. microdisca (f,b)* *Cacophryne cruentata (b)* *Rhacophorus javanus (b)* *Microphyla palmipes (b)*	*Rana cancrivora (f,b) R. limnocharis (f,b)* *Hylarana chalconota (f,b) H. nicrobariensis (f,b)* *Bufo melanostictus (f,b) B. biporcatus (f,b)* *Rhacophorus reinwardti (b)* *Polypedates leucomystax*
Arboreal		*Rhacophorus javanus (f,b)* *Philautus aurifasciatus (f,b)*	*Rhacophorus reinwardti (f,b)* *Polypedates leucomystax (f,b)* *Hylarana chalconota (f)*

TABLE 2.—*Altitudinal distribution of Tjibodas frogs and toads.*

Name of species	Cultivated land	Botanical garden	Primary rain forest		
	Rarahan 1,250 to 1,350 m	Tjibodas garden 1,350 m	Tjibeureum 1,600 to 1,700 m	Rawa denok 1,800 to 2,000 m	Lebak Saat 2,250 to 2,500 m
Bufo melanostictus	+	+	-	-	-
Bufo biporcatus	+	-	-	-	-
Bufo asper	+	-	-	-	-
Cacophyrne cruentata	-	-	+	+	+
Megophrys monticola	-	-	+	+	-
Leptobrachium hasselti	-	-	+	+	-
Microhyla achatina	+	+	-	-	-
Microhyla palmipes	-	-	+	-	-
Rana limnocharis	+	-	-	-	-
Rana cancrivora	+	-	-	-	-
Rana kuhli	-	+	+	-	-
Rana microdisca	-	-	+	-	-
Hylarana chalconota	+	+	-	-	-
Hylarana nicobariensis	+	-	-	-	-
Amolops jerboa	-	-	+	+	-
Philautus aurifasciatus	-	+	+	+	-
Rhacophorus javanus	-	+	+	-	-
Rhacophorus reinwardti	+	+	-	-	-
Polypedates leucomystax	+	+	-	-	-
Total number of species	10	8	9	5	1

Species which occur primarily in the rainforest are not found in the adjacent secondary growth (or cultivated land) or vice versa (Table 2). The Tjibodas Botanical Garden which is a large clearing carved out along the forest edge appears to be a transition zone between the cultivated land and the rainforest. This is shown by the presence of some finest species (*Rana kuhli, Philautus aurifasciatus,* and *Rhacophorus javanus*) and some lowland species (*Bufo melanostictus, Microhyla achatina, Rhacophorus reinwardti,* and *Polypedates leucomystax*) in the Tjibodas Botanical Garden (Table 2). The difference in altitudinal distribution of forest frogs in the primary rain forest of Mt. Gede is probably due to vegetation, vailability of food and of breeding sites (suitable water bodies for the development of tadpoles). The first two factors appear to be a consequence of altitude. In Tjibeureum (1,600 m) the vegetation is characterized by tall rasamala trees (*Altingia excelsa*), luxuriant growth of epiphytes, lianas, and undergrowth. At higher altitudes (2,000 to 2,500 m) except for *Podocarpus imbricata,* all other trees appear smaller, thinner and less abundant, with decrease in the number of plant species, but an abundance of lianas, and epiphytes (Doctors van Leeuwen 1945). The difference of vegetation is also reflected by the decrease in the number of species (Table 2): there are nine species at about 1,500 m, five species at 2,000 m, and only one species at 2,500 m above sea level. Out of the five species which survive the 2,000 m limit only *Leptobrachium hasselti* was found in the lowland of Java. Apparently the highest altitudinal limit of Javanese frogs is only around 2,000 m above sea level.

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ILLUSTRATIONS

PLATE 1.

FIG. 1. Forest floor within Tjibodas National Park. Habitat of *Microhyla palmipes* Boulenger near Gajonggong peat bog (1,600 m above sea level).

FIG. 2. Swift moving stream near Tjibeureum waterfall (1,700 m above sea level).

PLATE 2.

FIG. 1. Frothy nest of *Rhacophorus javanus* Boettger.

1

2

PLATE 1.

PLATE 2.

PUBLICATIONS AVAILABLE

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